CHLOROPHYLL *A* FLUORESCENCE MEASUREMENTS OF AN *ARABIDOPSIS* MUTANT, ALTERED IN THE γ-SUBUNIT OF THE ATP SYNTHASE, DISPLAY CHANGES IN NON-PHOTOCHEMICAL QUENCHING

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1. Introduction

In plants, the redox state of the γ-subunit of the ATP synthase is thought to play a role in the activation and the catalysis of the enzyme via the transmembrane electrochemical gradient, $\Delta\mu H^+$ (1). Gabrys et al. (2) have described a mutant called cfq (for coupling factor quick recovery) which was selected on the basis of absorbance changes at 518 nm. Ortiz and Ort (3) showed that the cfq has a point mutation (*atpC1*: E244K) in the γ-subunit of its ATP synthase. In this mutant, a larger ΔpH is expected for the activation of ATP synthase and a higher concentration of protons is expected to build up within the lumen. On the other hand, it has been suggested that the photoprotective mechanism against excess irradiance, that leads to "non-photochemical quenching" (NPQ) of chlorophyll *a* (Chl *a*) fluorescence, includes the effects of both protons and quenching by zeaxanthin formed from violaxanthin (4). In order to examine a possible relationship between ΔpH (or H^+s) and the NPQ, we have measured Chl *a* fluorescence from the leaves of wild type *Arabidopsis thaliana* and the cfq mutant.

2. Materials and Methods

Arabidopsis thaliana (ecotype Columbia) wild type and cfq mutant plants were grown at light intensities of 200 μmol quanta $m^{-2}s^{-1}$ for 6 weeks in a day night cycle of 12 h at 19° C. NPQ of Chl *a* fluorescence was measured at room temperature by a Pulse Amplitude Modulation device (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany) during exposure to light intensities of 20, 50, and 100 μmol quanta $m^{-2}s^{-1}$ Red actinic illumination (λ, 655 nm) was provided by 5 LEDs (H-3000 Stanley) focused onto the leaf surface (79 mm^2). Two other H-3000 LEDs, that emit 650 nm pulses, were used as measuring light. Leaf clip holder 2030-B equipped with micro-quantum sensor monitoring photosynthetically active radiation (PAR) was used. Chl *a* fluorescence was detected by a photodiode (BPY 12, Siemens) shielded by a RG-9 (Schott) long-pass far-red filter and a heat filter. All the fluorescence data were recorded in a time span of 5 min 20 s. The rate of data acqusition by the DA-2000 software in the preset program used (listed as run 3) was 10 ms/pulse. Run 3 measures Chl *a* fluorescence while calculating values of qN, a measure of NPQ, $[(F_m - F_m')/(F_m - F_o)]$; qP, photochemical quenching of fluorescence, $[(F_m' - F_t)/(F_m' - F_o)]$; and Y, photochemical yield, $[(F_m' - F_t) / F_m']$.

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Recording of data began 20 s following the onset of run 3 and the determination of F_o (minimal fluorescence yield of a dark adapted sample) and F_m (maximal fluorescence of dark adapted sample).

Chl *a* fluorescence transient, the OJIPS change (5), was measured using a shutter-less fluorometer (Plant Efficiency Analyzer, PEA, Hansatech, UK) which allows data accumulation starting at 40 μs. Chl *a* fluorescence transient was measured using 650 nm excitation light at intensities of 50, 250, and 750 μmol quanta $m^{-2}s^{-1}$.

3. Results and Discussion

The measurements of wild type and mutant fluorescence changes, acquired by the PAM 2000, are shown in Figure 1. Intact leaves, attached to the plant, were dark-adapted for 15 min prior to the recording of Chl *a* fluorescence. The fluorescence induction curves, F_t, and non-photochemical quenching, NPQ (as calculated by qN) show differences in the kinetics of Chl *a* fluorescence (*vide infra*) as well as NPQ between the wild type and mutant leaves. In the first 80 s of the measurement, the wild type displays a rise and a decline in qN. On the other hand, the cfq mutant shows a gradual increase of qN, markedly different from the wild type. In a study of the kinetics of energy-dependent quenching, so-called qE, of Chl *a* fluorescence of *Pothos* leaves, Joshi et al. (6) showed an increase in qE followed by a decline in the first 80 s in state I adapted plants, whereas in state II adapted plants, qE simply increased reaching a plateau at ~40 s. This apparent similarity in the qE of state II adapted *Pothos* and the qN of the cfq mutant of *Arabidopsis* requires further examination.

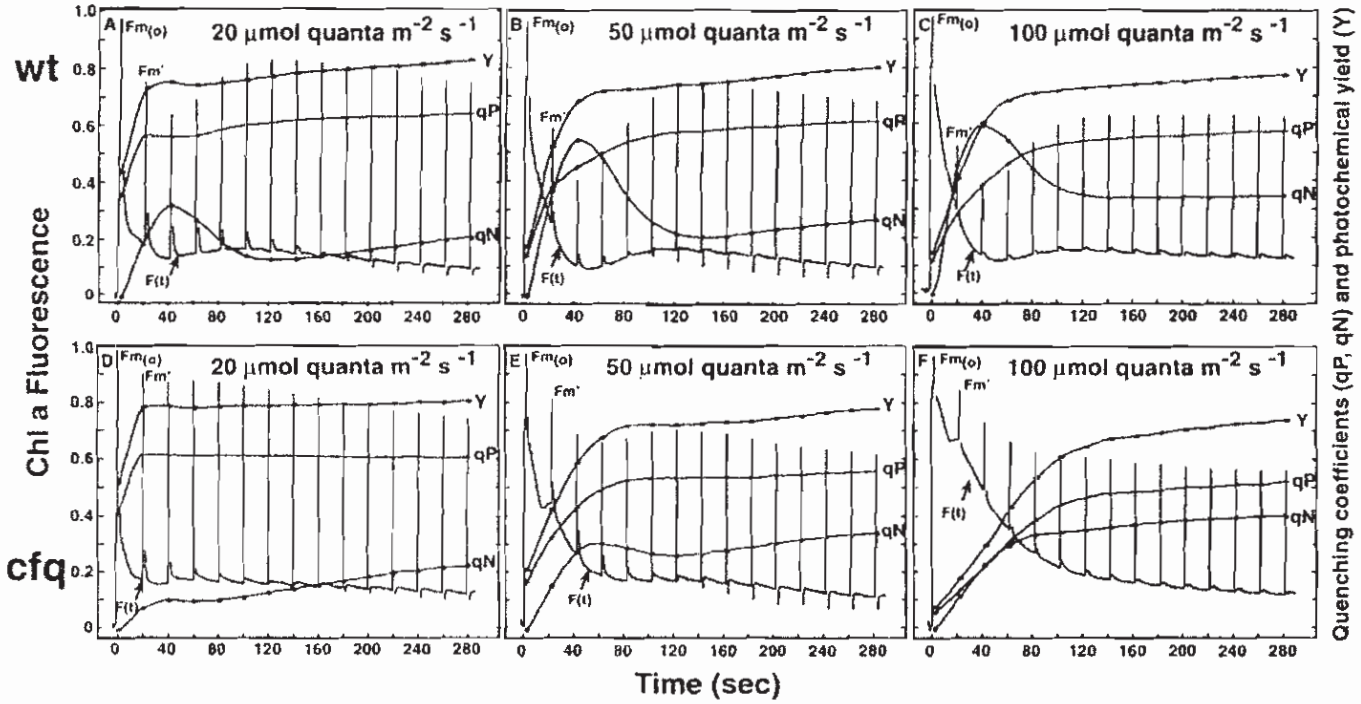

Figure 1. Chlorophyll *a* fluorescence (arbitrary units) with on-line calculation of photochemical quenching (qP), non-photochemical quenching (qN), and photochemical yield (Y), in intact, attached leaves of wild type and cfq mutant *Arabidopsis thaliana* plants. The recording was started at t=0 when the actinic, continuous light was turned on followed by an 0.8 s saturating pulse 2 s later. The initial spike of recording, $F_{m\,(0)}$ is nearly equal to the F_m measured in the dark prior to onset of actinic light (not shown). Following $F_{m\,(0)}$ determination, saturating pulses determined F_m' at intervals of 20 s.

At all three intensities of continuous light, 20, 50, and 100 µmol quanta $m^{-2}s^{-1}$, the wild type qN measurements differ from those of the cfq mutant, and as the light intensity is increased, the differences become more significant. This is further visualized by plotting F_m/F_m', also a measure of NPQ (7) as a function of time (Fig. 2A, 2B, 2C). These plots of F_m/F_m' confirm the observations on qN: the ratios of mutant to wild type F_m/F_m' (Fig. 2D) show greater effects at the higher intensities.

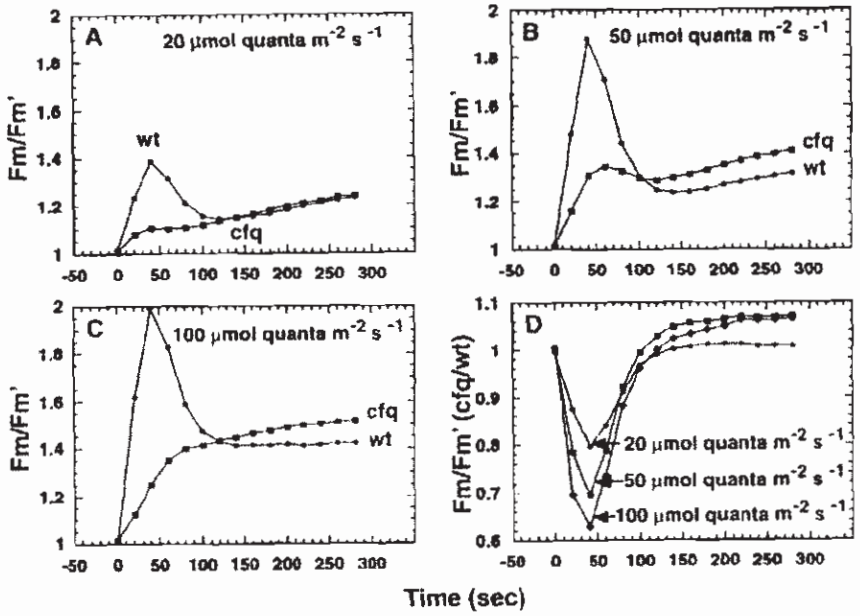

Figure 2. Fm /Fm' values for wild type and cfq mutant of *Arabidopsis thaliana* leaves at three different light intensities (A, B, and C). Panel D displays the ratios of F_m/F_m' values between cfq mutant and wild type. Intact, attached leaves were dark adapted for 15 min prior to measurements.

It is likely that the concentration of protons within the thylakoid lumen plays a role in the differences in the NPQ kinetics between the cfq mutant and wild type plants, since ΔpH is also involved in the regulation of the γ-subunit of ATP synthase (2). In all the measurements, the rise in NPQ of wild type plants to a higher level than that of the cfq mutant was quite unexpected. According to the present interpretation of this result, where higher NPQ is associated with greater lumen $[H^+]$ (4,7), the mutant appears to accumulate less, rather than more, protons within the lumen, especially in the initial 60 s of the measurement. Assuming the mutant thylakoid membrane does not leak protons (H^+), the interpretation of the data is apparently opposite to our expectation that the cfq mutant should have greater lumen acidity (2). However, this may be due to differences in the kinetics (i.e., time dependence) of the ΔpH between the wild type and the cfq mutant. The NPQ does become higher in the cfq than in the wild type at ~300 s at 100 µmol quanta $m^{-2}s^{-1}$.

Briantais et al. (8) have shown that, during fluorescence induction, the decay from P to S is linearly related to the acidification of the lumen. It follows that as the lumen is acidified Chl *a* fluorescence is quenched. Thus, we measured the fluorescence induction in the wild type and the cfq mutants (Fig. 3). Although there are no differences during the OJIP rise, the P to S decay from 1 to 10 s is slower in the cfq mutant than in the wild type (also see F_t curves in Fig. 1). This supports the conclusion that in this time scale the cfq mutant has lower lumen $[H^+]$ than in the wild type. Parallel kinetic measurements on 518 nm absorbance change, lumen pH, and Chl *a* fluorescence are needed to resolve the apparent paradox between the current interpretation of the NPQ and the P to S fluorescence and those based on the expectation from the γ-subunit mutation.

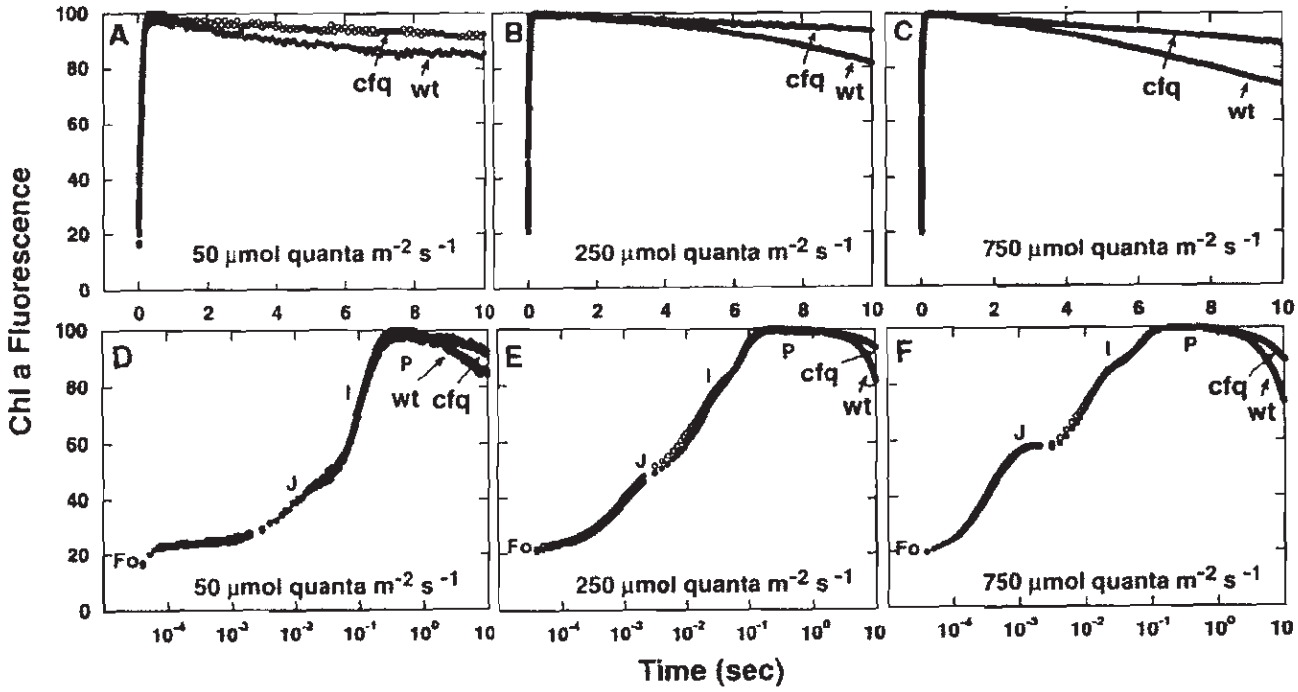

Figure 3. Normalized Chl *a* fluorescence transient curves of dark adapted wild type and cfq mutant of *Arabidopsis thaliana* on linear (top panels) and logarithmic time scale (bottom panels). Intact, attached leaves were dark adapted for 10 min prior to exposure at light (λ, 650 nm) intensities of 50, 250, and 750 µmol quanta $m^{-2}s^{-1}$.

Concluding Remark: In this paper, we show that the kinetics of non-photochemical quenching and the P to S fluorescence decay are quite different in the cfq mutant (γ subunit *atp C1*: E244K) from the wild type *Arabidopsis thaliana*. The relationship of these differences to changes in the kinetics of lumen acidification as well as to the so-called state changes need to be further examined before we can fully explain the observations presented here.

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