Femtosecond Transient Absorption Spectroscopy on the Light-Adaptation of Living Plants

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Abstract. The photoprotection reaction of the photosynthetic system under excessive sun light has been resolved for the first time by femtosecond absorption spectroscopy from the visible to near-infrared in intact leaves of Arabidopsis thaliana. The light-adaptation process was measured and a prominent non-photochemical quenching (npq) behavior located in photosystem II was observed. Among the various npq quenching mechanisms which have been discussed so far the most likely is the formation of chlorophyll-chlorophyll charge-transfer states which create a powerful energy dissipation pathway for the quenching.

1 Introduction

Photosynthetic systems in natural environment are exposed to a wide range of incident light intensities which may change within seconds. Without special photoprotective mechanisms photosynthetic systems would suffer severe photodamage in excessive sun light. As the most sensitive to photodamage especially photosystem II undergoes light-adaptation in order to safely dissipate the excess excitation energy as heat in a process called non-photochemical quenching [1].

2 Materials and methods

So far fluorescence techniques have been widely applied to study the in vivo response of plants and picosecond time-resolved fluorescence measurements are well established [2]. In contrast, in vivo femtosecond absorption measurements on intact leaves face a variety of severe technical obstacles: a) Scattering by coarse inhomogeneous structure inside leaves with their veins and chloroplast bodies virtually destroying laser beams, b) xy-scanning for averaging over a wider leaf area necessary to avoid local heating and photodamage, c) variations in the optical density from 0.1 to 2.0 in the leaf from spot to spot (sieve effects), d) production of oxygen bubbles leading to varying scatter during the measurements. All of these difficulties add to the problem that in vivo photosynthetic supercomplexes exhibit an extremely low annihilation threshold due to the large antenna sizes of the photosystems. Further challenges are provided by the fact that the photosystems can adapt to different photochemical states and by the requirement that the excitation pulses as well as the white light continuum (WLC) by themselves may already induce npq reactions during the

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measurement. This requires extremely low intensities in the order of a few µW average power also for the probe light continuum (WLC) and additional xy-scanning of the sample.

In order to compensate for the extraordinary scattering effects the detection system was reconfigured to resemble an open microscope collecting as much scattered photons out of the back of the leaf as possible. Test measurements showed that the scattered light indeed contains all the information needed to retrieve the true delta absorbance changes of the leaves. Furthermore to account for the high inhomogeneity in the leaf a discrimination window was dynamically controlled in real time during the measurements which rejects laser shots in areas with extremely high optical density for the veins and also atypical low optical density.

For excitation 60-fs pulses @620 nm and 3 kHz repetition rate with a pulse energy of about 1-2 nJ in a spot diameter of 100-200 µm were used (≈10^{13} ph/cm^2/pulse) on native fresh leaves from Arabidopsis thaliana at room temperature (22°C). For test purposes the full cycle of dark-light-dark adaptation on the same area of a single leaf of A. thaliana in each state after a 40 min interval for adaptation is monitored thus ensuring the integrity and full functionality of the leaf over the full measurement cycle. No degradation was observed. The integrity of the photosynthetic apparatus is further demonstrated by the fully intact oxygen evolution of the leaf. The npq state was induced by illumination with red light from an LED (625 nm, 500-1000 µE/m^2/sec).

Finally, by implementing a rapid scan technique for femtosecond spectroscopy, the measurement was extended to a real-time scanning of the npq development which allowed the full recording of the femtosecond kinetics in about 1-2 minutes, thus allowing a “slow motion” recording of the npq development with full femtosecond time and spectral resolution.

2 Results and discussion

Using a specialized optical and electronic design which corrects and accounts for the above-mentioned problems the npq processes have been revealed in intact leaves from Arabidopsis thaliana in vivo. The changes in the ultrafast kinetics can directly be observed in the raw data of the transient absorption decays (Figure 1). The long-lived decay components disappear when npq is induced and the overall decay kinetics occurs to be 2-3 times faster.

The results show that in fact the kinetics of PSII and PSI can be well distinguished and that npq mostly affects the PSII supercomplex in the wavelength range 675-695 nm. In the dark-adapted state a strong nanosecond lifetime component as well as 30-200 ps components were detected for PSII. In the light-adapted npq state the PSII lifetimes are strongly reduced, in particular the nanosecond component from the PSII reaction center disappears completely, being replaced by ca. 100-400 ps lifetimes.

Measurements in the near-infrared region up to 1100 nm show that in contrast to previous reports no involvement of carotenoid states as dissipation pathways of the excess light energy can be observed under npq conditions. In particular no carotenoid cation is formed as suggested by [3], rather a quenching by chlorophyll (Chl)/Chl charge transfer state formation is observed as was already proposed based on femtosecond transient absorption measurements on aggregated isolated light harvesting complexes II (LHC II) [4].

The real-time measurements of npq development show that no intermediate lifetime components between 400 ps and 2 ns emerge gradually during the npq transformation, i.e. there occurs no gradual down-shift of the long-lived PSII nanosecond lifetime toward shorter lifetimes. Rather a step-like switching of lifetimes from ns to 100-400 ps occurs. A switch was proposed by van Grondelle and coworkers [5]. However our data clearly indicate that the quenching pathway is established by Chl-Chl charge transfer (CT) states as intermediates rather than by carotenoid excited states [4]. The real-time measurement reveals further details of the transformation to npq e.g. during the first few minutes the quenching starts with the formation of a 100-200 ps component and then subsequently slows down producing lifetime components up to 400 ps.

Measurements on isolated LHC II trimers in a gel without detergent at 80K support the existence of Chl-Chl charge transfer states at about 700 nm, which are not present in non-quenched LHC II
complexes also in gel (with detergent) at 80K. As previously reported the Chl-Chl CT states are also formed at room temperature in LHC II aggregates within 5-10 ps and decay with about 200 ps [4]. At low temperature two Chl-Chl CT states can be distinguished by the rise times of ca. 15-30 ps and 150-300 ps while the decay of the latter is above 800 ps.

**Fig. 1.** Transient absorption decays of an intact leaf of *Arabidopsis thaliana* (w.t.) at various detection wavelengths for the short (left) and the long time range (right) and non-quenched state (grey) and light-adapted npq state (dark curves). Under npq conditions the non-decaying nanosecond component in the photosystem II wavelength range disappears entirely and is substituted by a ca. 200 ps component.

**References**