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Picosecond time-gated fluorescence imaging of Photosystem II particles and spinach leaves

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Introduction

Picosecond fluorescence of plant photosynthetic proteins has been widely used to study energy and electron transfer in isolated particles of Photosystem II (PS II), Light-Harvesting Complexes and other related systems. The distribution of fluorescence emitted from whole plant material has been imaged previously but so far this has involved measurement of the time-averaged fluorescence with little attention to the spatial variation of picosecond fluorescence components in the tissue.

Ultraviolet-B (UV-B) radiation is known to cause serious photoinhibition of plant and algal photosynthesis, (Tevini et al., 1989, Bornman et al., 1984) and is exacerbated by enhanced UV-B due to stratospheric ozone depletion. Here, we demonstrate the use of time-gated picosecond fluorescence imaging for the study of whole plant tissue and show that the technique can be used to investigate the effects of UV-B photoinhibition.

Materials and methods

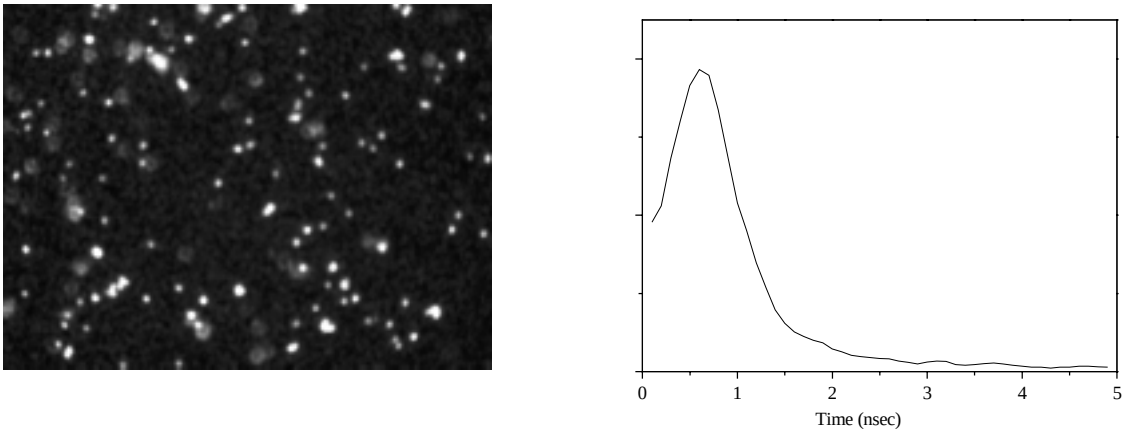
PS II membrane fragments were prepared according to standard procedures (Berthold et al.,1981) and spinach leaf tissue was used untreated. Picosecond time-gated fluorescence microscopy with 100 ps time resolution was performed using a gated microchannel-plate-intensified CCD camera system (LaVision Picostar) mounted on an optical microscope (Ziess Axiophot), a 60 ps 635 nm pulsed diode laser for excitation of the sample in the chlorophyll Q-band and a 675 nm bandpass filter in front of the camera to detect chlorophyll fluorescence and reject stray light. For measurements of the effects of UV-B inhibition, samples were irradiated with narrow linewidth (< 30 GHz) tunable UV (285 - 310 nm) from an intracavity frequency-doubled Rhodamine-6G ring dye laser system (Coherent 899) pumped by an argon ion laser (Coherent I90-6).

Results

We first demonstrated the technique of picosecond time-gated imaging of plant material by performing experiments on PS II membrane fragments. Membrane fragments dispersed in buffer on a surface give fluorescence images (Fig. 1a) that indicate that the fragments have a uniform shape and size distribution and that there is some aggregation. The picosecond fluorescence decay curves of BBY preparations (Fig. 1b) give amplitudes and decay times of 0.5 at 200 ps and 0.5 at 1 ns for open reaction centers (with acceptors) and 0.85 at 420 ps and 0.15 at 2.3 ns for closed

reaction centers (no acceptors). These are consistent with the decay curves obtained previously by Roelofs et al. (1991) from time-correlated photon-counting measurements of PS II suspensions. For chloroplasts, the resulting fluorescence images resembled those commonly obtained using conventional light microscopy and the picosecond decay curves were similar to those obtained for PSII fragments. Here we emphasize that time-gated fluorescence imaging (TGFI) is primarily a high-resolution imaging tool. While it provides picosecond time resolution, the time resolution is less than that achievable by other methods such as time-correlated photon-counting (TCPC). Therefore, the kinetic parameters obtained from TGFI may be less certain than those from TCPC. For TGFI with this time resolution (100 ps) it is only sensible to fit up to a maximum of 2 exponentials in view of the available number of data points.

Picosecond chlorophyll fluorescence imaging experiments were performed for scans of 5 ns with 100 ps resolution on precisely the same region of whole spinach tissue both before and after UV irradiation. Fig. 2a shows selected frames for both the control and UV-irradiated sample at 0 and 200 ps. The peak fluorescence at 0 ps shows localized and well-defined cellular structure in the case of the control but relatively diffuse and non-specific structures in the UV-irradiated case indicating significant UV-induced rupture of the tissue structure. The total fluorescence signal integrated over the whole image for this time (0 ps) is similar for the control and UV-irradiated cases because, although the fluorescence decay time can change, the chlorophyll emission cross-section is not significantly altered by UV irradiation. These UV-induced effects are also reflected in the images for later times, such as those at 200 ps. We have analyzed the fluorescence time decay profiles for each pixel in both the control and UV-irradiated experiments. The amplitudes and decay times are 0.8 at 450 ps and 0.2 at 840 ps for the control, and 0.9 at 400 ps and 0.1 at 860 ps for the UV-treated sample. Therefore, the UV-irradiated samples show faster fluorescence decay and a more random spatial distribution of fluorescence compared with untreated samples



(a) (b)

Fig. 1. Picosecond fluorescence image of a dispersion of PSII membrane fragments (a) and fluorescence decay at one of the pixels on the image (b)

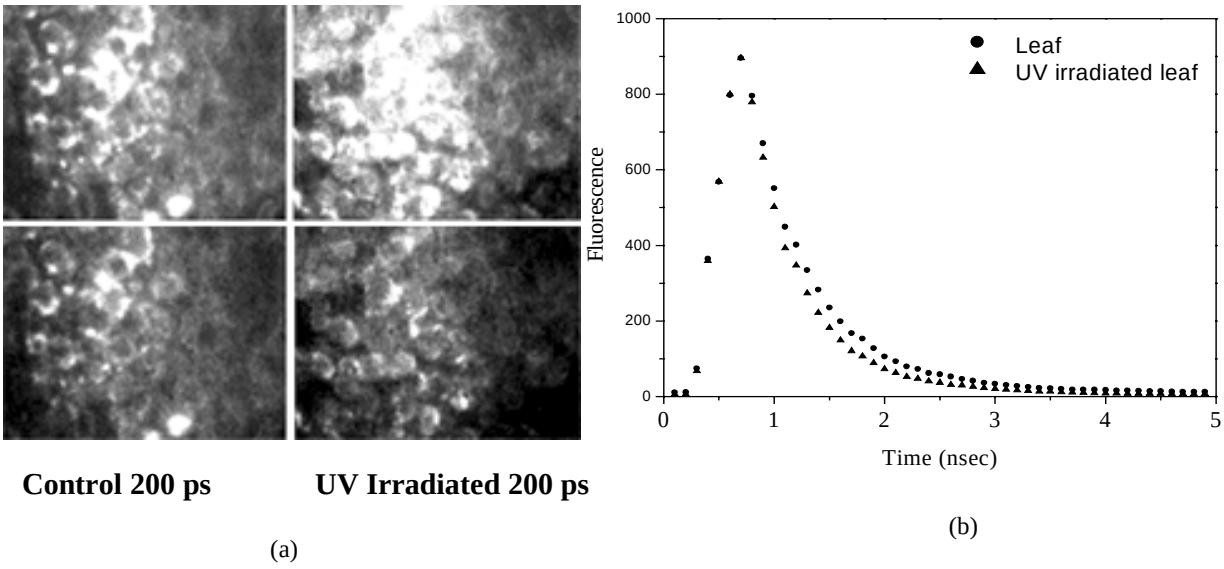


Fig. 2. Fluorescence images of spinach leaf without and with UV irradiation of 0.8mW at 300nm (a) and corresponding fluorescence decay curves (b)

Discussion

The changes in the chlorophyll fluorescence decay kinetics indicate UV-induced uncoupling of chlorophyll molecules in the light-harvesting antennae system. This uncoupling will adversely effect the energy reorganization in the light-harvesting systems and subsequent exciton transfer to the PS II reaction center which occurs in the intact photosynthetic unit. This damage to the antennae systems at both the molecular and cellular levels will lead to reduced photosynthetic activity of the organism when exposed to UV-B.

Acknowledgments

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