

Fluorescence lifetime measurement and imaging of chlorophyll in UV-stressed *Tetraselmis* microalgae *in vivo*

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Summary

Chlorophyll *a* fluorescence lifetimes were measured under both normal and UV-stressed conditions on the green microalgae *Tetraselmis in vivo*.

Introduction

The fluorescence lifetime is a very useful parameter in investigating biological materials on the molecular level, as it is mostly independent of fluorophore concentration [1]. The green algae *Tetraselmis* blooms in summer, and therefore its response to UV-irradiation is of particular interest. It is well known that both UV-A and in particular UV-B radiation reduces the growth rate and photosynthetic activity of phytoplankton and algae [2, 3]. We have measured the *in vivo* chlorophyll *a* fluorescence lifetime under both normal and UV-stressed conditions on *Tetraselmis*. There are several processes available for the chlorophyll molecules to return to a lower energy state, most prominently fluorescence, Förster resonance energy transfer (FRET) and photochemistry, all of which are in direct kinetic competition. This means that fluorescence and fluorescence lifetimes can be used to monitor non-fluorescent reactions and mechanisms of photosynthesis. Recent studies have shown that the main (usually out of two) lifetime component of *in vivo* chlorophyll *a* fluorescence is between 170-305 ps [4, 5, 6, 7]. It follows that if the cells reduce the photosynthetic activity, the fluorescence lifetime will increase. However, the photosynthetic apparatus is extremely complex, and several processes and mechanisms play a role in its protection under stress conditions. With this work, we aim to add knowledge to this very important field of research.

Results and Discussion

As shown in Table I, our measurements of chlorophyll *a* under normal conditions resulted in two separate lifetime components, τ_1 at 262 ps and τ_2 at 728 ps. The relative amplitude of the short component was 87 %. For UV-stressed conditions, the main (short) lifetime component increased significantly to almost 400 ps. This indicates that a strong quencher has been eliminated from the system. It is reasonable to attribute this to a partial or complete shutdown of photochemistry. We are already planning to test this by chemically shutting down the PSII reaction centres using the herbicide DCMU.

It is also shown in Table I that after keeping the same algae sample in darkness overnight, the fluorescence lifetime returned to normal values, indicating that the protection mechanisms triggered by the UV-radiation is reversible on a relatively short time scale. It is also interesting to note the time and amount of the UV-dose exposed to the samples. A full UV-dose for 2 hours was necessary to induce a change in the lifetimes, and a half dose did not trigger any changes at all for the same time period. Figure 2 shows images of the algae cells color-coded for fluorescence lifetime. It is clear that the lifetime is non-uniform throughout the cell, and the outer subregions typically show a shorter lifetime.

Table I. Chlorophyll *a* fluorescence lifetime data for normal conditions, UV-stressed conditions (2 hours under full UV-B, UV-A and PAR) and post UV-stressed conditions (UV-stressed cells had been dark adapted afterwards for 24 hours). The bottom row shows Chlorophyll *a* fluorescence lifetime data for half dose UV-stressed conditions (2 hours under 50% UV-B in addition to full UV-A and PAR). τ_1 is the short lifetime component, τ_2 is the long lifetime component and a_1 is the relative amplitude of the short lifetime component. Uncertainties are calculated as one standard deviation.

Condition	τ_1 [ps]	τ_2 [ps]	a_1 [%]
Normal	262 ± 31	728 ± 133	87 ± 5
UV-stressed	389 ± 40	984 ± 145	85 ± 6
Post UV-stressed	280 ± 42	886 ± 142	80 ± 5
UV-stressed (reduced UV-B)	256 ± 23	750 ± 120	84 ± 5

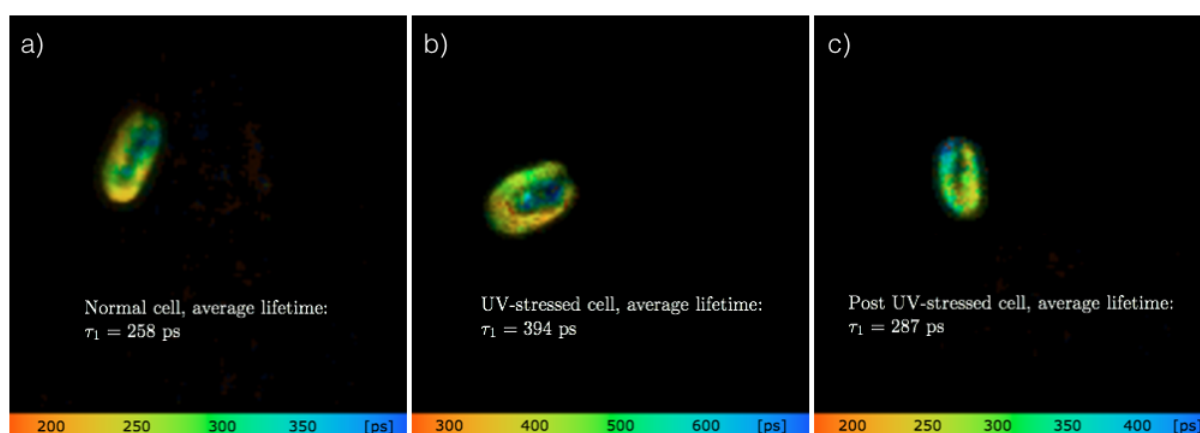


Fig 1. Chlorophyll fluorescence lifetime images of three separate *Tetraselmis* cells for three different conditions: a) Normal conditions, b) After 2 hours of UV-stress, c) Kept in darkness overnight.

Conclusions

We have shown that UV-stress of the green algae *Tetraselmis* increases the chlorophyll *a* fluorescence lifetime significantly, which is likely attributed to a partial or complete shutdown of photochemistry. However, the processes involved are extremely complex and not fully understood, as many proteins and pigments are involved. We aim to increase the knowledge about how photochemistry is regulated to UV-stress, and further investigations are ongoing.

References

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