Measurement of chlorophyll a fluorescence changes in single plant cells and cell regions in response to electrical stimuli

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Chlorophyll fluorescence is a sensitive indicator of photosynthetic energy conversion in higher plants, algae and bacteria. We applied microscopy-pulse amplitude modulation chlorophyll fluorometer (Microscopy PAM, Walz, Effeltrich, Germany) combined with an Axiovert 25 CFL inverted microscope (Carl Zeiss, Germany) to assess photosynthesis at the subcellular level by chlorophyll fluorescence using the saturation pulse method. The principle of saturation pulse method is as follows. When the weak measuring light is switched on, the minimal fluorescence yield, Fo, is assessed which is characteristic for the dark-adapted state with all PSII reaction centers (RC) being open. Upon application of a brief saturating light pulse, the fluorescence yield of the dark-adapted sample is transiently increased to its maximal level, Fm. The increase of fluorescence yield from Fo to Fm is called variable fluorescence, Fv. When actinic light is turned on, we assess the actual fluorescence F for light-adapted state with PSII RC partly closed; upon application of a saturation pulse, we assess the maximal fluorescence yield Fm' under illumination. Available information obtained with this approach includes the effective quantum yield of PSII, the so-called Genty parameter ?F/Fm' = (Fm'-F)/Fm' [1], the relative electron transport rate ETR = ?F/Fm' * PFD (where PFD is the photon flux density), minimal and maximal fluorescence, photochemical and non-photochemical quenching (PQ, NPQ respectively).

Cells of characean algae exposed to illumination arrange photosynthesis in coordinated spatial patterns. Using this method we investigated an impact of a single action potential (AP) on photosynthetic activities in different cell regions in Chara corallina cells. Generation of electrically-induced action potential (AP) in illuminated cells of Chara corallina was found to transiently suppress photosynthesis and give rise to NPQ. The effect of AP on NPQ, most evident at high Fm', was manifested as a strong long-lived drop of maximum fluorescence (Fm'). The sigmoid dependence of NPQ on PFD shifted after cell excitation towards lower fluence rates. Evidence was obtained that the shift in chlorophyll fluorescence after AP is due to the increase in ?pH at the thylakoid membranes.

[1] *Genty B., Britanis J.-M. & Baker N.R.* The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence //*Biochimica et Biophysics Acta* **990** (1989). Pp. 87-92.