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BRIEF COMMUNICATION

Photosystem 2 photoinactivation and repair in the *Scenedesmus* cells treated with herbicides DCMU and BNT and exposed to high irradiance

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Abstract

Changes in fluorescence parameters observed during irradiation of the *Scenedesmus* cells showed that photosystem 2 (PS2) photoinactivation in cells treated with phenolic PS2 inhibitor 2-bromo-3-methyl-6-isopropyl-4-nitrophenol (BNT) was significantly accelerated in comparison with control and DCMU-treated cells. Moreover, a negligible difference in the rate of PS2 photoinactivation in the absence and presence of chloramphenicol indicated that both DCMU and BNT blocked the PS2 repair process.

Additional key words: algae; 2-bromo-3-methyl-6-isopropyl-4-nitrophenol; chloramphenicol; chlorophyll fluorescence induction; 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Susceptibility to the irradiation-induced inactivation is a specific feature of the PS2 complex of oxygenic photosynthetic organisms. The PS2 photoinactivation (PS2PI) can be manifested by the irradiance-induced decline of photochemical activity measured as the rate of oxygen evolution or as the intensity of variable fluorescence. This decline is typically observed *in vitro* in isolated membrane preparations while *in vivo* it is much less apparent as it is counteracted by an efficient repair based on the degradation of the old copy and insertion of a new copy of the PS2 reaction centre protein D1 (for review see Aro *et al.* 1993). When D1 protein synthesis and

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Abbreviations: BNT - 2-bromo-3-methyl-6-isopropyl-4-nitrophenol; CAP - chloramphenicol; Chl - chlorophyll; DCMU - 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_o, F_m, F_v - constant, maximum and variable components of fluorescence; PS1 - photosystem 1; PS2 - photosystem 2; PS2PI - photoinactivation of photosystem 2.

incorporation are inefficient (in the presence of protein synthesis inhibitors, at low temperature or under extreme irradiance), PS2PI becomes obvious also *in vivo*. Under low irradiance the degradation of the D1 protein is blocked by the "classical" PS2-specific inhibitor DCMU but not by the phenolic compound BNT (Jansen *et al.* 1993). It has been argued that the difference can be explained by their distinct effects on the D1 conformation which influence the triggering of D1 degradation. However, Nakajima *et al.* (1996) suggest that the difference may arise from the ability of phenolic inhibitors to induce PS2 photodamage much faster compared with classical inhibitors. We studied the changes of fluorescence parameters in cells of the green alga *Scenedesmus* during the high irradiance treatment in the absence and presence of DCMU or BNT under functional or blocked chloroplast protein synthesis. The aim of the study was to clarify the effect of these herbicides on the processes of PS2PI and PS2 repair in algal cells under high irradiance.

A culture of the green alga *Scenedesmus quadricauda* (TURP.) BRÉB., strain Greifswald/15 was grown at 30 °C in a mineral medium and bubbled with air+2 % CO_2. For photoinhibitory treatments the culture was diluted to 30 g (Chl) m^{-3}, placed into an 18 mm thick cuvette, bubbled with air+2 % CO_2, and irradiated by 500 μmol(PAR) m^{-2} s^{-1}. The constant (F_0), maximum (F_M), and variable ($F_V = F_M - F_0$) components of chlorophyll (Chl) fluorescence were measured with a modulation fluorometer *PAM101* (*Walz*, Effeltrich, Germany). A modulated radiation source (red LEDs with the emission peak at 650 nm) providing less than 1 μmol(PAR) m^{-2} s^{-1} at 1.6 kHz was used for the determination of F_0 value while for the measurement of F_M the cell suspension was irradiated with "white light" of 500 μmol(PAR) m^{-2} s^{-1} until the signal reached maximum. Fluorescence of the control cells was assessed in the presence of DCMU added just before the measurement.

In the initial experiments we compared the effect of herbicides DCMU and BNT on the process of PS2PI. Therefore, the control and herbicide-treated *Scenedesmus* cells were irradiated at 30 °C in the presence of chloroplast protein synthesis inhibitor chloramphenicol (CAP, 50 g m^{-3} final concentration) in order to eliminate PS2 repair. The time course of PS2PI was followed by the measurement of fluorescence parameters after 2 min dark incubation.

In control cells the irradiation caused a gradual decrease of F_V/F_M (Fig. 1*A*, *open symbols*) which reached about 50 % after 2 h. In the initial hour only decrease of F_M was detected while during the second hour also rise of F_0 participated in the F_V/F_M decline (Fig. 1*D*). On the other hand, the photoinhibitory process in the presence of DCMU was typical by a fast initial increase of F_0, representing about 20 % of F_V, and by a following slow decrease of F_M (Fig. 1*B,E*, *open symbols*). PS2PI in the presence of BNT exhibited similar characteristics (Fig. 1*C,F*, *open symbols*) but the initial increase of F_0 was more extensive and reached nearly 50 % of F_V in 2 min. Then F_0 remained almost constant and F_V further declined due to the F_M decrease. This decrease was significantly faster compared with the same process in the presence of DCMU, and after 2 h of photoinhibition there was less than 10 % of F_V/F_M left. These results showed that in agreement with Nakajima *et al.* (1996) PS2PI in the BNT-treated cells of *Scenedesmus* was much faster than in the control cells or cells treated with the classical urea-type inhibitor DCMU.

By the measurement of fluorescence we could distinguish two different processes participating in the decline of F_V/F_M in herbicide-treated cells, and both were more pronounced in the presence of BNT: (*1*) the fast initial increase of F_0 reminding of a "very fast process" related to the modification of the PS2 acceptor side (Nedbal *et al.* 1990), and (*2*) the slower subsequent decrease of F_M, similar to the "fast process" according to Nedbal *et al.* (1990) or "irreversible damage" described by Kirilovsky *et al.* (1990).

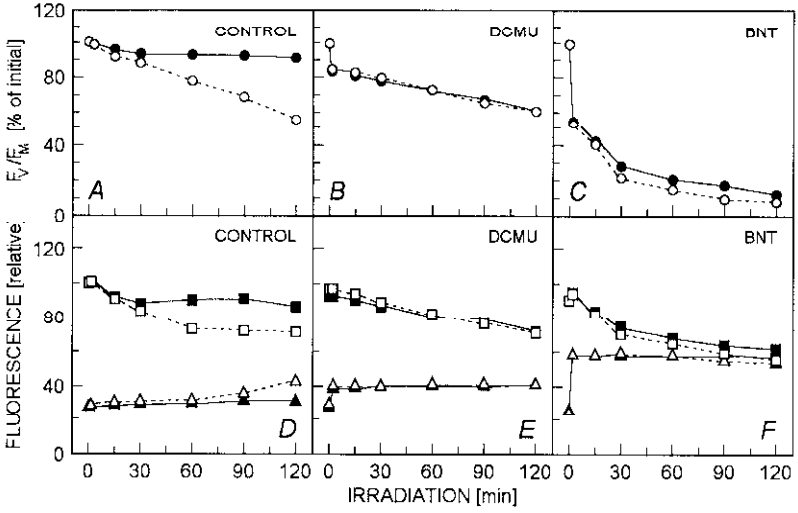

Fig. 1. Changes of F_V/F_M (*circles*), F_0 (*triangles*), and F_M (*squares*) during irradiation of the *Scenedesmus* control cells (*A,D*) and cells treated with 10^{-5} M DCMU (*B,E*) or 10^{-5} M BNT (*C,F*) in the absence (*closed symbols, solid lines*) or presence (*open symbols, dashed lines*) of CAP. Initial value of F_V/F_M was 0.705±0.015.

In order to assess an impact of both herbicides on the PS2 repair process we performed the same experiment in the absence of CAP. In the control cells no apparent PS2PI was observed (Fig. 1*A,D, closed symbols*) and comparison with the CAP-treated cells clearly documented the functional PS2 repair. On the other hand, a minimal difference between the fluorescence kinetics in the absence and presence of CAP in the herbicide-treated cells (Fig. 1*B,C,E,F*) was in accordance with the inhibition of the PS2 repair process by the herbicides. From our results it is difficult to establish what mechanism underlies this inhibitory effect. Herbicides may influence both the degradation of the old D1 copy (due to an interaction with its Q_B binding site) and the synthesis of a new one. The reduction of thioredoxin downstream of PS1 as well as a low level of ADP may be a prerequisite for the D1 synthesis in the green alga *Chlamydomonas reinhardtii* (for review see Mayfield *et al.* 1995). Since PS2 inhibitors cause an oxidation of the algal photosynthetic electron transport chain downstream of PS2 accompanied by the inhibition of photophosphorylation, I consider as a more probable explanation that herbicides block the D1 synthesis. This explanation is in agreement with results suggesting

existence of repair in cells of thermophilic cyanobacteria treated with DCMU (Šetlíková *et al.* 1984, Terjung *et al.* 1996). While Q_B binding site is similar in both eukaryotic algae and cyanobacteria, only cyanobacteria use plastoquinone as a common electron carrier for both the photosynthetic and respiratory electron transport chains. This feature allows a partial reduction of the cyanobacterial plastoquinone pool from the respiratory chain even under inhibited PS2, and, consequently, limited photophosphorylation and reduction of thioredoxin (needed for the D1 synthesis) can proceed.

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