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BRIEF COMMUNICATION

Inhibition of glutamine synthetase activity by phosphinothricin results in disappearance of the peak M2 of the chlorophyll fluorescence induction curve

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Abstract

The treatment of green algae *Chlorococcum lobatum* with the herbicide BASTA containing phosphinothricin lead to a significant decrease in the level of peak M2 of the chlorophyll fluorescence induction curve. This agrees with the suggestion that glutamine synthetase activity affects this region of the induction curve.

Additional key words: *Chlorococcum lobatum*; green algae; herbicide BASTA.

The slow phase of the chlorophyll (Chl) fluorescence induction consists of several characteristic peaks with maxima labelled as P, M (M1), and M2 (Govindjee 1995) (see Fig. 1). In contrast to the earlier transients, the peak M2 occurring at about 100 s of irradiation (Yamagishi *et al.* 1978) is studied very little. It is suggested that the activity of the ATP-dependent glutamine synthetase may contribute to the rise in the Chl fluorescence yield to the maximum M2 (Moskvin *et al.* 1998). This enzyme takes part in nitrogen assimilation and catalyzes the conversion of glutamate plus ammonia to glutamine (Knaff and Hirasawa 1991). A temporarily enhancing consumption of ATP by the glutamine synthetase, after the beginning of the operation of the glutamine synthetase-glutamate synthase cycle, may lead to decrease in the activity of the Calvin cycle. As a result, the Chl fluorescence intensity increases. The peak M2 coincides with time of both the maximum in the level of 3-phosphoglycerate and the minimum in the level of ATP (Quick and Horton 1986). The photochemical component of fluorescence quenching also declines during the S2-to-M2 transition (Quick and Horton 1984).

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The glutamine synthetase can be inhibited by the herbicide phosphinothricin (Krieg *et al.* 1990). This circumstance offers the opportunity to confirm the assumption that the changes in Chl fluorescence intensity after the minimum S2 can be attributed to the activity of glutamine synthetase. The aim of research presented here was to verify whether phosphinothricin affects the magnitude of the peak M2. It can be useful not only for better interpretation of this peak but also for a practical application of the Chl fluorescence induction method as a tool to test the sensitivity of plants and algae to phosphinothricin-containing preparations.

Pure culture of the unicellular green algae *Chlorococcum lobatum* (Korschikoff) Fritsch et John was grown on Bold's medium (1N BBM) (Arce and Bold 1958). Algal stocks on the agar media were maintained in a growth chamber (temperature 20-22 °C, irradiance 80 W m^{-2}, 12 h photoperiod). For the measurements the algae were added to water (control) or water solutions of the commercial herbicide BASTA (*Hoechst*, Frankfurt/M., Germany) containing phosphinothricin as the active compound. The samples were stored in the dark for 30 min, then Chl fluorescence induction curves were recorded using a laboratory-made single-beam fluorometer. The Chl fluorescence was excited with blue radiation of a mercury lamp passing through an *SZS-3* glass filter, which has a maximum of transmission around 480 nm. The actinic irradiance was 25 W m^{-2}. The fluorescence intensity was measured at 685 nm by the device consisting of an *FEU-79* photomultiplier, an *F4223* analog-to-digital converter, and a personal computer. Time intervals for recording Chl fluorescence induction curves were as follows: (*1*) 0-55 ms with discretization time of 110 μs, (*2*) 0.055-2.805 s with discretization time of 11 ms, and (*3*) 2 805-500 s with discretization time of 1 s.

In a typical Chl fluorescence induction curve of non-treated *C. lobatum* cells (only the second and third time intervals for the recording of values are presented in Fig. 1), the peak M2 is well pronounced. It can be characterized by means of the F_{M2}/F_{S2} ratio. F_{M2} and F_{S2} are Chl fluorescence intensities at reaching the maximum M2 and the minimum S2, respectively. The presence of phosphinothricin in the suspension of algae gave rise to a significant decrease in the F_{M2}/F_{S2} ratio (Table 1). The

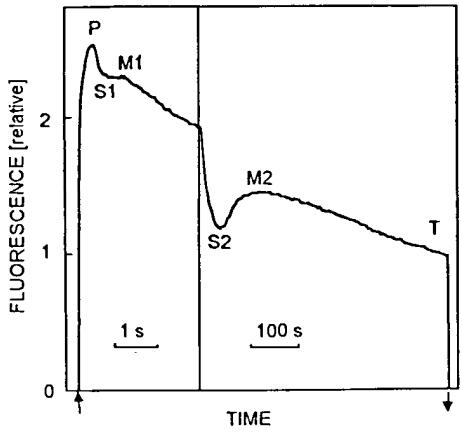

Fig. 1. Time course of light-induced changes in the chlorophyll fluorescence yield in dark-adapted *Chlorococcum lobatum* cells. The arrows indicate begin (↑) and end (↓) of irradiation.

Table 1. The effect of phosphinothricin, the active compound of the herbicide BASTA, on parameters of the chlorophyll fluorescence induction curve of the green alga *Chlorococcum lobatum*. Each value is the mean ± S.E. based on the five independent experiments. Different superscript letters in a column denote significantly different means at $p<0.05$. For explanation see the text.

Phosphinothricin [mM]	F_{M2}/F_{S2}	t_{M2} [s]	F_P/F_T
0	1.20 ± 0.02^a	121 ± 5^a	2.58 ± 0.05^a
0.3	1.19 ± 0.01^a	117 ± 5^a	2.50 ± 0.07^{ab}
1.5	1.15 ± 0.01^b	89 ± 3^b	2.40 ± 0.04^b
3.0	1.11 ± 0.01^c	70 ± 6^c	2.20 ± 0.05^c
7.5	1.04 ± 0.02^d	61 ± 5^c	2.01 ± 0.04^d
15.0	1.02 ± 0.01^d	40 ± 4^d	1.90 ± 0.07^d

magnitude of observed changes depended on the concentration of the herbicide. The concentration required for 50 % inhibition, I_{50}, was 3.0±0.2 mM (mean±S.E., $n = 5$). The peak M2 was almost eliminated as a result of the treatment with 15 mM phosphinothricin.

The presence of BASTA in the suspension of algae lead to a decrease in value of the parameter t_{M2}, the time between the onset of irradiation and reaching the peak M2 (Table 1). At the same time, there were no noticeable changes in the fast phase of the Chl fluorescence induction (before reaching the peak P) as well as in the kinetic of the P-S1-M1 transient (values not shown).

The results described here allow to conclude that the ATP-dependent glutamine synthetase actually plays an important role in the formation of the peak M2 of the Chl fluorescence induction curve. Inhibition of this enzyme activity by phosphinothricin limits the consumption of the ATP by the glutamine synthetase-glutamate synthase cycle. Under these conditions, the activity of the Calvin cycle is not diminished. Apparently, that is why Chl fluorescence does not increase at that time and the peak M2 is less pronounced or not observed at all.

In addition, the F_P/F_T ratios were determined. F_P and F_T are the levels of Chl fluorescence at reaching the peak P and at 500 s after the onset of the irradiation, respectively. The higher value of the F_P/F_T ratio in the control (Table 1) indicated that the contribution of the nitrogen metabolism to variable Chl fluorescence quenching after peak P is significant. The decrease in F_P/F_T ratio was probably induced partly by ammonia accumulation in the presence of herbicide.

Similar results were also obtained for other green algae, *Bracteacoccus medionucleatus* Bischoff et Bold and *Chlorella vulgaris* Beijerinck (values not shown). All algae species investigated are typical for the algal flora of soils in Europe (Ettl and Gärtner 1995). Therefore the application of the commercial preparations containing phospinothricin can result in a negative effect on the soil ecosystem.

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