

Different sensitivity of photosynthetic electron transport of plant species to lichen acids

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Introduction

The lichens are the unique symbiont of algae and fungi in nature. Usually, specific pair of lichen photobiont (algae) and lichen mycobiont (fungi) form the thallus. Majority of the lichen photobionts (about 60 %) belong to the genus *Trebouxia*, unicellular green algae (Ahmadjian 1993), and the major lichen mycobionts are ascomycete (Poelt 1973). The lichen mycobiont produces the lichen-specific secondary metabolites such as lichen acids from glucose and ribitol, photosynthetic products of lichen photobionts. However, the research on the ecological and physiological roles of these secondary metabolites are very limited. After Inoue et al. (1987) reported the inhibition of oxidizing side of P680 in PSII in spinach by lichen acid, no similar research was published until we reported the more detailed characterization of the inhibition in photosynthetic electron transport by the lichen acids in spinach (Endo et al. 1998). Barbatic acid, one of the most abundant lichen acids, primarily inhibited at the reducing side of P680, possibly Q_b, but also at the oxidizing side of P680, suggesting Y_z as the target. Because we found the different inhibitory activity among a variety of lichen acids, we have been also interested in characterizing the differences in the sensitivity among different species of photosynthetic organisms to lichen acids. Here we report that PSII of cultured tobacco cells, *Chlamydomonas reinhardtii* and lichen photobionts showed different sensitivity to lichen acids.

Materials and Methods

Plant materials and cell culture –Cultured tobacco cells (*Nicotiana tabacum* cv. Samsun NN NI:wild type, B and D: atrazine-tolerant strains (Sato et al.1988) were cultured photomixotrophically under fluorescent lamps at 50 μmol m⁻² s⁻¹ in Linsmaier-Skoog medium (Linsmaier and Skoog 1965) on a rotary shaker (80 rpm) at 25°C. Cells at their logarithmic phase of growth (14 or 15 day after inoculation) were filtered through 1 mm stainless mesh to eliminate large cell aggregates and were used for measurement. *Chlamydomonas reinhardtii* c-9 (IAM cell collection, Institute of Molecular and Cellular Bioscience, The University of Tokyo) was cultured photoautotrophically under fluorescent lamps at 200 μmol m⁻² s⁻¹ in the medium of Orth et al. (1966) on a rotary shaker (100 rpm) at 25 °C in 2 % CO₂. Cells at their logarithmic phase of growth (4 or 5 day after inoculation) were used for measurement. The three species of lichen photobionts (*Trebouxia impressa*, *Trebouxia* sp. and *Trebouxia excentrica*) were isolated from *Ramalina crassa*, *Ramalina litoralis*, and *Cladonia aggregata*, respectively for this study and were cultured photomixotrophically under fluorescent lamps

at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *Trebouxia* medium (Deson et al. 1960) on a rotary shaker (100 rpm) at 25°C . Cells at their logarithmic phase of growth (11 or 12 day after inoculation) were used for measurement.

Lichen acids - Usnic acid was purchased from Wako medicine Co., Ltd (Osaka). and used after recrystallization. Barbatic acid from *Cladonia aggergata*, diffractaic acid and evernic acid from *Usnea longissima*, salazinic acid from *Ramalina crassa* were isolated according to the method of Yamamoto et al. (1995).

Measurement- Chlorophyll fluorescence was measured with a PAM2000 chlorophyll fluorometer (Walz, Effertrich, Germany). The cells ($5\mu\text{g Chl/ml}$) were suspended in the buffer containing certain amount of lichen acids for 30 min and its chlorophyll fluorescence was measured. Fluorescent parameters, $((\text{Fm}-\text{Fo})/\text{Fm})$ and $(\text{Fm}'-\text{F})/\text{Fm}'$ were measured in the dark and under light (8 and $48 \mu\text{mol m}^{-2} \text{s}^{-1}$), respectively.

Results and discussion

Effect of barbatic acid on the cultured tobacco cells

$(\text{Fm}-\text{Fo})/\text{Fm}$ was measured to evaluate electron transport at the oxidizing side of P680, and $(\text{Fm}'-\text{F})/\text{Fm}'$ for that at reducing side of P680 as described previously (Endo et al. 1998). As Fig 1. shows, barbatic acid (BA) inhibited the photosynthetic electron transport activity in tobacco cells. As previously shown in spinach thylakoid membranes, higher concentration of BA decreased $(\text{Fm}'-\text{F})/\text{Fm}'$ more severely than $(\text{Fm}-\text{Fo})/\text{Fm}$; i.e. BA inhibited photosynthetic electron transport at reducing side more than oxidizing side.

The half inhibition concentration of the reducing side of P680 was about $50 \mu\text{M}$. The half inhibition concentration of the oxidizing side could not be determined due to the low solubility of BA.

When *C. reinhardtii* and three species of the lichen photobionts, *Trebouxia* were incubated with BA, no significant inhibition, was observed both in the oxidizing side and reducing side of P680 even at $100\mu\text{M}$ (data not shown). This result clearly indicated that the sensitivity to BA was different between the higher plant (tobacco) and the algae (photobionts of lichens and *C. reinhardtii*).

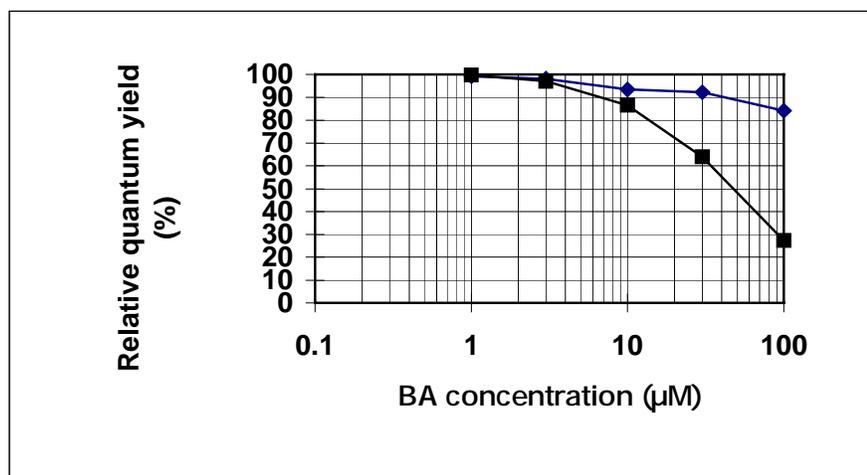


Fig. 1 Inhibition by barbatic acid (BA) of photosynthetic activity of PSII in tobacco cells was estimated from Chlorophyll fluorescence. \blacklozenge ; $(\text{Fm}-\text{Fo})/\text{Fm}$, \blacksquare $(\text{Fm}'-\text{F})/\text{Fm}'$. Values are shown as relative to the control (no addition of BA). Control values of quantum yield of PSII are follows; $(\text{Fm}'-\text{F})/\text{Fm}' = 0.44$, $(\text{Fm}-\text{Fo})/\text{Fm} = 0.71$

Effects of diffractaic acid, evernic acid, usnic acid and salazinic acid

Whereas BA was most inhibitory lichen acid in spinach thylakoid membranes, we examined the effects of other lichen acids. Interestingly, some lichen acids such as diffractaic acid inhibited both the oxidizing side and reducing side of P680 in *C. reinhardtii*. Because the half inhibition concentration of diffractaic acid as well as usnic acid and evernic acid for the inhibition of oxidizing side and reducing side in *C. reinhardtii* was almost same, these lichen acids primarily inhibited the oxidizing side in *C. reinhardtii*; The half inhibition concentration in the reducing side and in the oxidizing side were 130-250 μM and 140-350 μM for these lichen acids, respectively. On the other hand, three species of the lichen photobionts showed no inhibition at the oxidizing side and reducing side of P680; The half inhibition concentration in the reducing side and in the oxidizing side were more than 600 μM for usnic acid, or more than 1000 μM for diffractaic acid, evernic acid and salazinic acid.

These data indicated that the lichen acids inhibited the photosynthesis of various photosynthetic organisms at cellular level, although plant species showed different sensitivity to each lichen acid. The mechanism of different sensitivity of plant species to lichen acids is not clear. Interestingly, lichen photobionts were tolerant to all lichen acids examined. The *allelopathy* is known for some plant species which secrete chemicals to inhibit the growth of a nearby plant. Lichen acids may function as allelopathic agents of lichen to inhibit the growth of other plants, since lichen acids hardly inhibit the photosynthesis of lichen photobionts whereas inhibit that of tobacco and *C. reinhardtii*. The ecological survey is needed to clarify the role of the lichen acids as allelopathic agents.

To get more information about the primary site of inhibition, atrazine-tolerant tobacco cells were used to evaluate the effect of BA. BA inhibited the reducing side of P680 more strongly in atrazine –tolerant strains (B,D) than wild type (the half-inhibition concentration in wild type and atrazine-tolerant strains (B,D) were 47 μM , 2 μM , 1.3 μM , respectively). Atrazine-tolerant tobacco cells have mutated D1 protein which probably reduce the atrazine binding at Qb site of PSII. Similar increased sensitivity of atrazine-tolerant cells to phenol herbicides is also reported (Shigematsu et al. 1988), suggesting that lichen acids may function as phenol type.

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