Inhibition of PSII in Atrazine-Tolerant Tobacco Cells by Barbatic Acid, a Lichen-Derived Depside

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Received July 19, 2005; Accepted August 24, 2005

In atrazine-tolerant tobacco cells with Ser to Thr mutation at the 264th amino acid of PsbA polypeptide in photosystem II (PSII), electron transport around the secondary quinone acceptor (Q_B) site was inhibited to a greater extent by barbatic acid than in wild-type cells. Further characterization suggests similar mode of action of barbatic acid and phenyl-type herbicides.

Key words: atrazine-resistance; lichen acid; inhibition of photosynthesis; photosystem II (PSII); psbA gene

Lichens are unique symbionts of algae and fungi in nature. Usually, a specific pair of lichen photobionts (algae) and lichen mycobionts (fungi) form the thallus. The majority of lichen photobionts (about 60%) belong to the genus Trebouxia, unicellular green algae, and the major lichen mycobionts are ascomycete. Lichen mycobionts synthesize lichen-specific secondary metabolites such as lichen acids from glucose and ribitol, photosynthetic products of lichen photobionts. To date, about 400 lichen acids have been reported. Lichen acids are roughly classified into three types; i.e., depside, depsidone, and dibenzofuran. So far, the inhibitory activity of lichen acids on the growth of microorganisms, lichens, moss and plants have been studied.

For example, Nishitoba et al. showed that eight depsides and orcinol derivatives isolated from Usnea longissima inhibited the growth of lettuce seedlings. On the other hand, the characterization of their mode of action is limited. Inoue et al. reported that usnic acid inhibited the oxidizing side (up-stream of the electron flow) of P680 of PSII in spinach chloroplasts. More recently, we showed that barbatic acid (BA), a major lichen acid, and related depsides inhibited electron transport around 161st tyr (Yz) in PsbA polypeptide, on the oxidizing side of P680 and around the secondary quinone acceptor (Q_B) on the reducing side (down-stream of the electron flow) of P680. Because the mode of dual inhibition of the oxidizing and reducing sides is not known, we examined the inhibitory activity in atrazine-tolerant tobacco cells that have a mutant Q_B site in psbA (D1) protein. Since atrazine-tolerant tobacco showed higher sensitivity to BA than the wild type, we discussed the possibility that barbatic acid functions as the phenol type herbicide dinoseb (Fig. 1).

Abbreviations: BA, barbatic acid; Chl, chlorophyll; DCMU, dichlorophenyldimethylurea; DMBQ, 2,6-dimethylbenzoquinone; F, steady state Chl fluorescence under light; Fm, maximum yield of Chl fluorescence at closed PSII center induced by a pulse of saturating light measured after adaptation in the dark; Fm', Fm under light; Fo, minimum yield of Chl fluorescence at open PSII center; IC_{50}, half inhibition concentration; P680, reaction center chlorophyll of PSII; PSII, photosystem II; Q_A, primary quinone acceptor in PSII; Q_B, secondary quinone acceptor; Yz, tyrosine-161 in D1 polypeptide
We used atrazine-tolerant (cell lines B and D) and wild-type cultured tobacco cells (cell line NI; *Nicotiana tabacum* cv. Samsun NN), which were maintained photomixotrophically under fluorescent lamps at 50 μmol m⁻² s⁻¹ in modified Murashige and Skoog medium, as described previously. Cultured cells in the logarithmic phase of growth were used for the experiments. Cells were homogenized in buffer A with 2 mM MgCl₂, 20 mM KCl, 50 mM HEPES, and 0.4 M sucrose (pH 7.6) with a mixer (Ace Homogenizer AM-8 Nihonseiki, Japan) at 15,000 rpm 3 times for 3 s, then the chloroplasts were precipitated by sequential centrifugation (350 × g for 2 min, then, 2,000 × g for 10 min). After the chloroplast envelope was disrupted by osmotic shock, the thylakoids were sedimented at 2,000 × g for 15 min and resuspended in the buffer A supplemented with 10 mM NH₄Cl. Barbatic acid was isolated from *Cladia aggregata* by the method of Yamamoto et al.Ｄosineb was purchased from Wako Pure Chemical (Osaka, Japan). Chlorophyll fluorescence from the thylakoids (5 μg chlorophyll/ml) was measured with a PAM 2000 chlorophyll fluorometer (Walz, Effeltrich, Germany).

In a previous study, we developed a novel method to identify inhibition sites in PSII using quenching analysis of chlorophyll fluorescence. We used the (Fm’-F)/Fm’ value measured under weak light (5–30 μmol m⁻² s⁻¹) as the indicator of inhibition on the reducing side of P₆₈₀ in PSII. On the other hand, we used Fm measured in the dark as an indicator of inhibition on the oxidizing side of P₆₈₀ when Fo was constant or slightly increased. Further study showed that the site of inhibition on the oxidizing side was around the Yz residue whereas the site on the reducing side was around the Q₈₀-binding site.

In this study, we measured the effects of barbatic acid (BA) on electron transport in thylakoid membranes of two lines of atrazine-tolerant tobacco cells using chlorophyll fluorescence analysis. As shown in Fig. 2, the higher concentration of BA decreased (Fm’-F)/Fm’ in both wild-type and atrazine-tolerant tobacco cells. The inhibition profiles indicate that half inhibition concentrations of BA for electron transport around Yz were higher than 100 μM in both wild-type and atrazine-tolerant cells. In contrast, BA inhibited electron transport around Q₈₀ more strongly than around Yz in terms of decreases in (Fm’-F)/Fm’, with inhibition of electron transport around Q₈₀ in atrazine-tolerant tobacco cells at lower concentrations of BA than in the wild type. A comparison of IC₅₀ in inhibition around Q₈₀ showed that the atrazine-tolerant cells B and D were 24 and 36 times more sensitive to BA respectively than the wild type.

The exact binding mode of phenol-type herbicide on the reducing side of D1 has not been elucidated. The binding site is used both for phenol-type herbicides and for others such as phenylurea-, triazine-, triazinone- and uracil-type herbicides. Our previous model for phenylureas might provide the basis for the binding model for phenol-type herbicides. A strong hydrophobic interaction is sufficient for herbicide binding.
References