Note

Effects of Batatasin III and Its Analogs on Gibberellic Acid-Dependent α-Amylase Induction in Embryoless Barley Seeds and on Cress Growth

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Received March 6, 1998

The effects of batatasin III and its analogs on gibberellic acid (GA₃)-dependent α-amylase induction in embryoless barley seeds and on cress root-growth were examined. Batatasin III was most effective as an important factor of α-amylase induction at 4 × 10⁻⁴ M, but its potency was low compared with that of abscisic acid. In the cress test, p-hydroxybibenzyl had high activity.

Key words: batatasin III; dihydrostilbene derivatives; GA₃-dependent α-amylase induction; abscisic acid-like activity

Dormancy is one of the important physiological processes of some plants and its chemical control is very interesting. In many plants, abscisic acid (ABA) has been described to participate as an important factor in the process. In the Chinese yam plant (Dioscorea batatas Dence), batatasins isolated as endogenous growth regulators were reported to be essential factors in the dormancy of bulbs. Among them, batatasins III (1), IV, and V have dihydrostilbene structures similar to luinicular acid, which was isolated as a growth inhibitor in the liverwort, Lunularia cruciata; and suggested to have hormone-like activity in primitive green plants. Already, Hashimoto and Tajima examined the effects of batatasins and their analogs in lettuce seed germination, lettuce hypocotyl elongation, and wheat coleoptile section elongation tests, well-known assay systems for ABA analogs, and they observed that at the concentrations higher than 10⁻⁴ M, batatasin I was least effective, but the other batatasins and the analogs were almost comparable in inhibitory activity. Further, several papers have reported that some aromatic analogs had ABA-like activities. Since we have been interested in another ABA-like activity of batatasins, we investigated the effects of batatasin III and its analogs on gibberellic acid (GA₃)-dependent α-amylase induction in embryoless barley seeds, which is also a well-known assay system for ABA analogs.

Batatasin III was prepared by a similar method to that described by Hashimoto and Hasegawa. Analogs 2–4, 6, and 8–11 were synthesized similarly from the corresponding substituted benzaldehydes via the Wittig reaction, and following catalytic reduction. Trihydroxy compounds 5 and 7 were obtained from 4 and 6 by demethylation of their methoxy groups with pyridine-HCl, respectively.

The effects of the synthetic compounds on GA₃-induced α-amylase activity in embryoless barley seeds (Hordeum vulgare L. cv. Kyushu Hadaka 3) were measured by the procedure described in the previous paper. As the result of the test (Table 1), batatasin III caused 68% inhibition of α-amylase induction by GA₃ at the concentration of 4 × 10⁻⁴ M. At 10⁻⁴ M, the inhibitory rate was 22%. Compound 6, the para-isomer of batatasin III, had a comparable inhibitory effect, 65% and 26% at 4 × 10⁻⁴ M and 10⁻⁴ M, respectively, but the other compounds tested were less effective. Comparing

### Table 1. Effects of Batatasin III (I) and Its Analogs (2–11) on GA₃-Dependent α-Amylase Induction in Embryoless Barley Seeds and on Root-Growth of Cress

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<tr>
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<th>Conc. 4 × 10⁻⁴ (M)</th>
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<tr>
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<td>α-Amylase Induction</td>
<td>L. sativum Root growth</td>
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<tr>
<td>No.</td>
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<td>3</td>
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<td>11</td>
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</table>

a Three of the embryoless barley seeds (Hordeum vulgare L. cv. Kyushu Hadaka 3) were placed in a 50-ml screw vial containing 1.0 ml of a test solution (GA₃, 5 × 10⁻⁴ M, streptomycin, 0.5 mg, and an appropriate amount of a test compound) and were incubated at 30°C for 48 hr. The amount of reducing sugars in the vial was measured by the Somogyi-Nelson method. All experiments were duplicated.

b Twenty cress seeds (Lepidium sativum) were placed on one sheet of Toyo No. 2 filter paper soaked with a test solution in a Petri dish (9 cm i.d.) at 25°C under long-day conditions (16 hr light, 8000 lux). After 4 days, the root length was measured. All experiments were duplicated.

c Not tested.

d Tested at 5 × 10⁻⁴ M.

e Tested at 1.5 × 10⁻⁴ M.

f Tested at 10⁻⁴ M.
the activity of batatasin III with those of 2, 3, and 4, it was found that methylation of hydroxy groups of batatasin III decreased the activity, while the trihydroxy derivatives 5 and 7 were less effective than their corresponding monomethyl isomers, batatasin III and 6. The nature of the substituents and their positions on the ring might be important to the activity. Although batatasin III was most effective among tested compounds, its potency seemed to be two orders of magnitude lower than that of ABA, which was highly inhibitory to GA$_3$-induced $\alpha$-amylase biosynthesis even at $10^{-6}$ M.

Furthermore, we examined the effects of the synthetic compounds on germination and root-growth of cress, Lepidium sativum, according to the general procedure.\textsuperscript{20} All compounds tested had no inhibitory effect on germination of cress seeds at the concentration of $4 \times 10^{-4}$ M (data not shown). However, they inhibited the root-growth of the plant considerably. Batatasin III caused 70% and 39% inhibition at the concentration of $4 \times 10^{-4}$ M and $10^{-4}$ M, respectively. This potency seemed to be consistent with the results of lettuce hypocotyl elongation and wheat coleoptile section elongation tests by Hashimoto \textit{et al.}\textsuperscript{40} They observed strong inhibitory activity at higher than $10^{-4}$ M, along with slight but reproducible inhibitory activity at low concentrations. In this experiment, $p$-hydroxybibenzyl, 11, inhibited the root-growth of cress almost completely at $5 \times 10^{-4}$ M and 64% at $1.5 \times 10^{-4}$ M and it seemed to be more potent than the other compounds tested.

In both experiments, it appears that dihydrostilbenes with hydroxy and methoxy groups had low ABA-like activities. Already, it has been found that the six-membered ring of ABA can be replaced by an aromatic residue without much alteration to the hormone activity.\textsuperscript{8, 10} The compounds carrying an aromatic side chain, which would structurally resemble both ABA and lunularic acid, were also found to be active in inhibiting seed germination.\textsuperscript{9} The compounds tested here might be considered as mimics in which both the ring moiety and the side chain of ABA were replaced with aromatic groups, although their potency were far inferior to that of ABA. Since aromatic compounds of the carboxylic type were more active than the corresponding phenolic type of compounds, probably due to not only their similar molecular shapes to ABA, but also acidity,\textsuperscript{12} dihydrostilbene derivatives with a carboxylic group such as lunularic acid might be expected to have more activities in the tests.

Recently, Tanno \textit{et al.}\textsuperscript{14} reported the identification of endogenous ABA from yam bulbils (Dioscorea opposita and D. japonica) and suggested its probable participation in their dormancy. As the effect of other batatasins has not been investigated in this experiment, the role of them in the yam plant could not be discussed in detail. However, batatasin III had low ABA-like activities and might be expected to affect the physiological process, cooperating with other similar compounds.

References