Bioactive Gibberellin and Its Metabolism in Shoots of Phaseolus vulgaris

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Evidence is presented for gibberellin A1 (GA1) being bioactive per se in regulating the shoot elongation of Phaseolus vulgaris. It is also suggested that the lower response of cv. Masterpiece (dwarf) to GA1 than of cv. Kentucky Wonder (normal) was not due to any difference in GA1 metabolism, but to a defect in the sequence of events from the reception of GA1 to elongation.

Key words: GA1, metabolism; prohexadione; shoot elongation; Phaseolus vulgaris; dwarfism

Evidence has accumulated that gibberellin A1 (GA1) is bioactive per se in regulating the shoot elongation of many higher plants.1-41 In the preceding paper,51 we showed that the early-13-hydroxylation GA biosynthetic pathway leading to GA1 mainly operated in the vegetative shoots of Phaseolus vulgaris L., and suggested that GA1 may be bioactive per se in regulating the shoot elongation of P. vulgaris L. We also showed that the endogenous levels of GAs in the shoots of cv. Kentucky Wonder (KW; normal) were similar to those of cv. Masterpiece (MP; dwarf), and that the response of MP to GA1 was lower than that of KW. Based on these results, we speculated that the dwarfism of MP was not due to any shortage of GA1 (the putative bioactive GA), but to its low ability to respond to GA1. In this study, we attempt to prove that GA1 is bioactive per se in regulating the shoot elongation of P. vulgaris L. In addition, the metabolism of [13C,1H]GA1 in KW seedlings is compared with that of MP seedlings to speculate the cause for the low response of MP to GA1.

Evidence supporting GA1 to be bioactive per se was obtained by examining the effects of prohexadione on the stem elongation of P. vulgaris L. Prohexadione is a plant-growth regulator that effectively inhibits 2β-hydroxylation (the deactivation step) as well as 3β-hydroxylation (the activation step) in the biosynthesis of GAs.6,7,11 The results are shown in Table 1. For the treatment without prohexadione, exogenous GA30 exhibited weak activity toward stem elongation, but by treating with prohexadione, the stem elongation of the control plants and of the plants treated with GA30 was inhibited to the level of ca. 85% of the control plants without the prohexadione treatment. The growth inhibition by prohexadione of the control and of the GA30-treated plants is considered to have been due to its inhibition of the conversion of endogenous and/or exogenous GA30 to GA1 (3β-hydroxylation; the activation step). On the other hand, treatment with prohexadione clearly enhanced the stem elongation of the plants treated with GA1 at 0.1 μg/plant. This enhancement was probably due to inhibition of the conversion of GA30 to GA1 (2β-hydroxylation; the deactivation step). These results indicate that GA1 was bioactive per se in regulating the shoot elongation of P. vulgaris L. With an application of GA1 at 1 μg/plant, the enhancement by treating with prohexadione was not significant. This may have been due to nearly saturated elongation by the application of a high dose of GA1.

Concerning the causes for the low response of MP to exogenously applied GA1, several possibilities could be considered. Ota51 has reported that the response of MP to GA1, which was applied by spraying, was also low, and that GA1 applied to cutting leaves of MP was rapidly converted into an unknown GA-like substance, while most of GA3 applied to leaves of the highly responsive cultivars of P. vulgaris L. remained unmetabolized. Based on this information, the metabolism of GA1 in shoots of MP was compared with that of KW to examine the relationship between the metabolism of exogenously applied GA1 and the low response of MP to GA1.11 [13C,1H]GA1 was applied to the shoot apex of each 8-day-old seedling, and two days after the treatment, 10 seedlings were harvested, separated into five parts and extracted with methanol. Most of the radioactivity was recovered from the third and fourth internodes, including the shoot apices (KW, 98.2%; MP, 98.2%), and therefore, the metabolites of [13C,1H]GA1 from the third and fourth internodes were further analyzed. The methanol extract was subjected to silica gel chromatography to give a GA free-acid fraction and GA conjugate fraction. The GA free-acid fraction from KW was further purified by octadecylsilane (ODS)-HPLC to give two radioactive fractions corresponding to those of GA1 and GA8. After converting to the methyl ester-trimethylsilyl ether (Me-TMS), [13C,1H]GA1 and [13C,1H]GA8 in each GA free-acid fraction were identified by full-scan GC-MS and gas chromatography-selected ion monitoring (GC-SIM), respectively. Similar results were obtained with the GA free-acid fraction of MP. The radioactive components of the GA conjugate fractions of KW and MP were indicated to be a [13C,1H]GA3-glucoside-like substance, a [13C,1H]GA1-glucosyl ester-like substance and a small amount of [13C,1H]GA1 by the retention time with an ODS-HPLC analysis. The tentative assignment of these metabolites in the GA conjugate fractions was also supported by the results of a TLC analysis. These results

<table>
<thead>
<tr>
<th>Prohexadione</th>
<th>Control</th>
<th>GA1</th>
<th>GA10</th>
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<tbody>
<tr>
<td></td>
<td>0.1 μg</td>
<td>1.0 μg</td>
<td>0.1 μg</td>
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<tr>
<td>0 μg</td>
<td>29.7 ± 1.1</td>
<td>33.3 ± 1.9</td>
<td>46.3 ± 1.3</td>
</tr>
<tr>
<td>80 μg</td>
<td>25.1 ± 0.9</td>
<td>43.6 ± 2.5</td>
<td>46.2 ± 3.2</td>
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</tbody>
</table>

Each value represents the mean length ± S.E. (mm) of the second internodes from 8 plants.

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** Abbreviations: GA, gibberellin; GA1, gibberellin A1; GC-SIM, gas chromatography-selected ion monitoring; KRI, Kovats retention index; KW, cv. Kentucky Wonder; Me-TMS, methyl ester-trimethylsilyl ether; MP, cv. Masterpiece; ODS, octadecylsilane.
are summarized at Table II, showing that the components of the main metabolites from $^{13}$C, $^3$H]GA$_3$ and their amounts in KW were similar to those in MP. This result means that KW and MP exhibited almost the same metabolic patterns of $^{13}$C, $^3$H]GA$_3$ in the third and fourth internodes (the elongated part) including the shoot splices. This suggests that the low response of MP to GA$_3$ was not due to any difference in GA$_3$ metabolism, but to a defect in the sequence of events from the reception of GA$_3$ to elongation.

**Experimental**

**Bioassay.** KW seedlings were grown under continuous fluorescent light of 270 μmol m$^{-2}$ s$^{-1}$ at 25°C. When their second internodes were about 0.5 cm long, the shoot apex of each seedling was treated with prohexadione, which had been prepared from prohexadione calcium, and/or GA$_3$, or GA$_{19}$ in a 10-μl drop of 50% aceton. To test the GA activity of the *P. vulgaris* seedlings treated with prohexadione, each GA$_3$ was applied 12 h after the treatment with prohexadione. The length of the second internode of each seedling was measured 6 days after the treatment.

**Application and extraction.** $^{13}$C, $^3$H]GA$_3$ (1 μg/plant) in a 10-μl drop of 50% aceton was fed to the shoot apex of each 8-day-old seedling grown under continuous fluorescent light of 270 μmol m$^{-2}$ s$^{-1}$ at 25°C, and the resultant seedling was grown under the same conditions. After two days, ten seedlings from each treatment were harvested, and the third and fourth internodes containing the shoot apices, first and second internodes, leaves, petioles and roots were separately extracted with methanol. The radioactivity of the aliquot of the methanol extract was determined by a liquid scintillation counter (LSC-2000, Aloka, Tokyo, Japan).

**Purification.** The methanol extract was chromatographed in a silica gel column (2 g: Wako gel C-300, Wako Pure Chemical Co., Japan) which was eluted in 20-ml fractions with an increasing ethyl acetate content in n-hexane in 10% steps, and then eluted with methanol. All the eluent contained 0.1% acetic acid. A fraction eluted with 50–100% ethylacetate and methanol gave a GA-free-acid fraction and a GA conjugate fraction, respectively. The GA free-acid fraction was injected into a Senshu-Pack ODS 425SD HPLC column (250 mm × 10 mm i.d.) which eluted 30% aceton containing 0.1% acetic acid at the flow rate of 3 ml/min. A major radioactive fraction and minor one were eluted at tR 21–23 min (fraction L) and tR 8–10 min (fraction H), corresponding to the tR values for GA$_3$ and GA$_{19}$, respectively.

**GC-MS and GC-SIM.** A Hewlett-Packard 5890A gas chromatograph coupled to a Hewlett-Packard 5970B series mass selective detector were used. The operating conditions and conversion were used the same as those applied before. After the conversion, fractions L and H were analyzed by full-scan GC-MS and by GC-SIM, respectively. In the GC-SIM analysis, ions at m/z 595, 580, 536, and 449 were monitored to detect $^{13}$C, $^3$H]GA$_3$-MeTMS. A solution of Paraflin was co-injected with each samples to determine the Kovats retention index (KRI).$^{11}$ $^{13}$C, $^3$H]GA$_3$-MeTMS, KRI: 2664; MS m/z (relative intensity): 507 (M$^+$, 100), 492 (21), 449 (33), 377 (37), 314 (15), $^{13}$C, $^3$H]GA$_3$-MeTMS, KRI: 2817; MS m/z: 595 (M$^+$, 100), 580 (20), 536 (24), 449 (82).

**Analysis of the GA conjugate fractions.** These fractions were injected into a Senshu-Pack ODS 425SD HPLC column as already described. GA$_3$-glucoside, GA$_3$-glucosyl ester, and GA$_3$ were eluted at tR 6.8–8.1 min, 18–20 min and 21–23 min, respectively. GA$_3$-glucoside, GA$_3$-glucosyl ester and GA$_3$ migrated by TLC at R$_f$ 0.05, 0.35, and 0.25, respectively, when a chloroform–methanol (3:1, v/v) solvent system was used, and at R$_f$ 0.15, 0.40, and 0.65, respectively, with a chloroform–methanol–acetic acid–H$_2$O (45:15:3:2, by volume) system.

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**References**