The Occurrence of Auxin-induced Pectin Methylation in Plant Tissues [1, 2]

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Treatment of coleoptile tissues with indoleacetic acid (IAA) lead to an enhanced incorporation of methyl groups into both the cold-water-soluble uronic acids (C-Pectin) and the hot-water-extractable fraction of the cell wall (HW-Pectin). This promotion of pectin methylation is one of the most extensively studied of the biochemical effects of auxin (4, 5, 7, 8, 9). There are 2 reasons for this particular attention. First, it is one of the few biochemical effects which has been shown to occur in response to auxin rather than to cell elongation. This is indicated by the fact that IAA still enhances pectin methylation even when all cell elongation is osmotically inhibited (9). Secondly, it was thought that this reaction might be important in the loosening of the cell wall and, thus, in auxin-induced cell elongation (8,9). This possibility has been eliminated by the finding that auxin-induced cell elongation can occur even when auxin-induced methylation is completely blocked by ethionine (5).

Although this auxin-induced pectin methylation does not lead to cell elongation, it may still be important in the growth or development of cells. If this is so, one might expect to find this particular biochemical effect in most primary plant tissues. At present, auxin-induced pectin methylation has only been found in one highly specialized organ, the coleoptile of Avena and maize (4). The following investigation was carried out in order to determine whether auxin-induced pectin methylation is of common occurrence in plant tissues. Selected for study were tissues in which elongation is promoted by IAA (Avena coleoptile, maize coleoptile and mesocotyl, Helianthus hypocotyl, and pea epicotyl), insensitive to IAA (Avena leaf), or inhibited by IAA (pea root).

Materials and Methods

Plant Material. Seedings of *Avena sativa* var. Victory were grown as described earlier (5). When the coleoptiles were 2.5 to 3.25 cm in length, the coleoptile and leaf were carefully separated. Seedlings of *Zea mays* var. Asgrow were grown in a similar manner. Coleoptiles 2 to 4 cm in length were selected and the leaf was removed. Mesocotyls were used which were 2 to 4 cm in length. They were severed from the coleoptile and leaf by an incision at the node. Seeds of *Helianthus annus* var. Mammoth Russian and *Pisum sativum* var. Alaska were soaked for 2 hours in distilled water, planted in moist vermiculite, and allowed to germinate at 25° under a dim red light. Helianthus hypocotyl sections were taken from 5-day-old seedlings after the apex had been removed by an incision at the base of the crook. Seven-day-old pea seedlings were used in which the third internode was 2 to 4 cm. in length. The apex was removed in a similar manner. Pea roots were obtained as follows. Alaska pea seeds were surface sterilized with Chlorox, and after soaking for 2 hours in distilled water, were laid out on moist cheesecloth which was suspended on a screen over distilled water. After 80 hours in the dark, roots 2.0 to 3.5 cm in length were collected.

The apical 2 mm of each tissue were excised and the next 10 mm were then removed as one section. The sections were collected in lots of 50 (maize coleoptile and mesocotyl, Helianthus hypocotyl), 100 (pea epicotyl and root) or 150 (Avena coleoptile and leaf). This was sufficient plant material to give 25 to 50 mg of dry cell wall.

Incubation. Each lot of sections was placed in 10 ml of 0.0025 M K-Maleate buffer (pH 4.8) which contained, as the methyl donor, 1.0 μc of L-methionine-methyl C^{14} (2 mc/mM). The appropriate solutions also contained 28.5 μM IAA (5 ppm). In the pea root experiments the concentration of IAA was 5.7 μM. The sections were incubated for 3 or 4 hours under a dim red light. At the end of the incubation the length of the sections was measured. Each treatment was run in duplicate; each experiment was repeated at least three times.

Preparation of the Pectin Fractions. The procedure for isolation of the pectin fractions has been described in detail elsewhere (5). Briefly, the tissues were ground in ice-cold buffer and the cell contents were separated from the walls by filtration. Microscopic examination showed that less than 2 % of the cells remained intact after homogenization. Cold-water-soluble uronic acids (C-Pectin) were precipitated from the filtrate with cold 70 % ethanol. The washed precipitate was resuspended and the methyl ester and uronic acid content of this suspension was determined.

Cell wall pectic substances were extracted in 2 steps. First, the walls were extracted twice for 30 minutes with 3 ml of boiling water. This extract contained the hot-water-soluble pectins. (HW-Pectin). The residual pectin (R-Pectin) was removed by extracting the walls twice for 30 minutes with 3 ml of boiling 0.05 N HCl. The methyl ester and uronic acid content of each extract was determined.

Methods of Analysis. The content of radioactive methyl groups was measured by determining the loss of radioactivity from each fraction upon saponification for 1 hour with 0.1 N NaOH. The freed methanol was lost during the drying of the sample for counting. Radioactivity was determined with sam-

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ples on planchets counted under a Micromil window tube in an atmosphere of Q gas.

Slight differences in sample size have been corrected for by expressing the results for each fraction as 10^3 cpm per 100 mg dry cell wall. Since the uptake of methionine into the tissues was auxin-insensitive and almost identical for all the tissues studied, no correction was made for the slight differences in uptake which were measured.

The uronic acid (AUA) content of each fraction was determined by the borate-sensitized carbazole reaction of Bitter and Muir (3). Although the AUA content of a particular fraction varied considerably between tissues, no effect of auxin could be detected in any fraction in these experiments. The results have not been expressed in terms of AUA since in the absence of more detailed knowledge of the exact chemical nature of each fraction it is not possible to determine how much of the apparent AUA content is actually due to uronic acids and how much is due to contaminating sugars.

Nature of the Pectic Fractions. The pectic substances have been arbitrarily separated into 3 fractions on the basis of their solubility. All 3 fractions contain methylated galacturonic acid but the fractions are certainly not chemically homogenous (7). For example, the HW-Pectin fraction of Avena coleoptile walls can be fractionated into protein-bound and nonprotein-bound pectic fractions (unpublished). However, it can be said with some certainty that the C^{14}-methyl groups found in the HW-Pectin and R-Pectin fractions of each of the tissues are esterified to polygalacturonic acid. This is shown by the fact that most of these methyl groups are released by treatment of the fractions with pectin methylesterase. The nature of the C-Pectin fraction is more uncertain. The fact that polygalacturonase releases galacturonic acid from the C-Pectin fraction of Avena coleoptiles (1) and pea stems (6) suggests that pectin is present in this fraction.

Results

The effect of IAA on the methylation of the C-Pectin and HW-Pectin fractions in monocotyledonous tissues is shown in table I. A correlation is apparent between the effects of auxin on elongation and the methylation of these 2 fractions. In the 3 tissues (Avena coleoptile, maize coleoptile, maize mesocotyl) in which auxin enhances elongation, methylation is promoted 15 to 50 % by auxin. Neither elongation nor methylation is sensitive to auxin in the Avena leaf.

The effect of IAA on the methylation of the C-Pectin and HW-Pectin fractions in dicotyledonous tissues is shown in table II. In contrast to monocotyledonous tissues, no significant auxin-induced increase in pectin methylation is found in any of the 3 dicotyledonous tissues tested. The methylation of C-Pectin appears to be slightly enhanced by auxin in Helianthus hypocotyl and pea epicotyl tissues. While this effect has been noted in most of the experiments with these 2 tissues, it is not significant at the 5 % level. No effect of auxin on the methylation of HW-Pectin was found with either tissue. The methylation of both pectic fractions in the pea root is strongly inhibited by auxin.

The methylation of the R-Pectin fraction (tables I and II) is either insensitive to IAA (Avena coleoptile, maize coleoptile and mesocotyl, Helianthus hypocotyl, pea epicotyl) or is inhibited by IAA (Avena leaf, pea root).

Discussion

The transfer of methyl groups from methionine to the 3 pectic fractions occurs in all of the tissues tested. But auxin-induced methylation is limited to the C-Pectin and HW-Pectin fractions of elongating monocotyledonous tissues. It appears that the sensitivity of the pectin methylating system to auxin may

Table I

The Effect of IAA on Pectin Methylation in Monocotyledonous Tissues

Sections were incubated with 1 μc of methionine-methyl-C^{14}, with or without IAA (28.5 μM). Pectic fractions were isolated and the MeOH-C^{14} content was determined as detailed in text.

Tissue	Incubation time hrs	IAA	MeOH-C^{14} incorporation* C-Pectin	HW-Pectin	R-Pectin	Elongation %
Avena coleoptile	3	+	15.2	30.9	26.4	9.2
		−	11.5	26.4	25.7	3.5
Maize coleoptile	4	+	11.3	16.7	23.2	19.5
		−	7.6	12.6	24.3	6.5
Maize mesocotyl	4	+	5.2	6.6	10.8	16.2
		−	4.3	5.0	11.2	6.4
Avena leaf	3	+	3.8	2.8	4.3	1.4
		−	4.0	2.9	5.2	1.4

* 10^3 cpm/100 mg dry cell wall.

Table II
The Effect of IAA on Pectin Methylation in Dicotyledonous Tissues
Sections were incubated 4 hours with 1 μc of methionine-methyl-C^{14}. Pectic fractions were isolated and MeOH-C^{14} content was determined as detailed in text.

Tissue	IAA μM	MeOH-C^{14} incorporation* C-Pectin	HW-Pectin	R-Pectin	Elongation %
Helianthus hypocotyl	28.5	30.6	58.8	29.4	21
	0	28.3	58.4	31.0	7
Pea epicotyl	28.5	2.84	18.1	17.8	13
	0	2.64	18.4	17.5	5
Pea root	5.7	5.5	53.6	48.4	2.4
	0	5.8	68.0	61.7	5.4

* 10^3 cpm/100 mg dry cell wall.

be a taxonomically-correlated character. The fact that this sensitivity has only been found in monocotyledonous tissues suggests that this character is limited to the class Monocotyledoneae. However, since all of the monocotyledonous tissues tested to date are members of the grass family, auxin-induced methylation may be restricted to the family Gramineae. A much greater variety of tissues must be tested in order to determine the exact taxonomic limitations of auxin-induced methylation. It should be noted that another auxin-effect, resistance to herbicidal concentrations of 2,4-D, was first thought to be a characteristic of monocotyledonous tissues but that more extensive investigation has shown this to be an oversimplification (2).

The occurrence of auxin-induced pectin methylation depends on several factors in addition to the species of the plant. This auxin-effect has only been found in tissues in which elongation can also be promoted by auxin. Thus methylation is enhanced in the coleoptile but not the leaf of *Avena sativa* L. The demonstration by Ordin et al. (9) that the auxin-effect could occur in Avena coleoptile sections even in the absence of elongation indicates that it is the potential for elongation rather than elongation itself which is correlated with the auxin-induced methylation. A second factor that must be noted is that different varieties of a single species may differ in sensitivity to auxin. For example, the methylation of HW-Pectin in maize mesocotyls is promoted by auxin in the varieties Asgrow and Barbacue Hybrid (unpublished) but insensitive to auxin in the variety Wisconsin 335 Hybrid (4).

The restricted occurrence of auxin-induced pectin methylation makes it unlikely that this process plays any important role in the growth or development of cells. It seems more likely that the enhanced methylation occurs simply because the methylating system in certain tissues is sensitive to one of the biochemical changes which occur in tissues treated with auxin.

Summary

1. The effect of IAA on the transfer of methyl groups from methionine to cold-water-soluble (C-Pectin), hot-water-soluble (HW-Pectin) and acid-soluble pectic substances (R-Pectin) has been examined in 4 monocotyledonous and 3 dicotyledonous tissues.

II. Auxin-induced methylation of C-Pectin and HW-Pectin is restricted to monocotyledonous tissues whose elongation is promoted by auxin. The methylation of these 2 pectic fractions is not enhanced by auxin in dicotyledonous tissues or in non-elongating monocotyledonous tissues.

III. The methylation of R-Pectin is insensitive to auxin in all tissues tested.

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