Short Communication

Oxindole-3-acetic Acid, an Indole-3-acetic Acid Catabolite in Zea mays

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ABSTRACT

A prior study (13) from this laboratory showed that oxidation of exogenously applied indole-3-acetic acid (IAA) to oxindole-3-acetic acid (OxIAA) is the major catabolic pathway for IAA in Zea mays endosperm. In this work, we demonstrate that OxIAA is a naturally occurring compound in shoot and endosperm tissue of Z. mays and that the amount of OxIAA in both shoot and endosperm tissue is approximately the same as the amount of free IAA. Oxindole-3-acetic acid has been reported to be inactive in growth promotion, and thus the rate of oxidation of IAA to OxIAA could be a determinant of IAA levels in Z. mays seedlings and could play a role in the regulation of IAA-mediated growth.

Indole-3-acetic acid in the endosperm of germinating seedlings of Zea mays is formed and destroyed at about 96 pmol/h (3). Only 12 pmol/h of this turnover was accounted for by catabolic decarboxylation following application of [1-14C]IAA to the endosperm. The major catabolite of IAA, which retains the carboxyl group, has recently been identified as OxIAA by demonstrating that [1-14C]IAA is catabolized to [1-14C]OxIAA2 by Z. mays endosperm (13).

Most prior studies of IAA catabolism examined the in vitro oxidation of IAA by purified horseradish peroxidase (8) and by tissue homogenates (cf. 5, 16). Other workers studied the degradation of IAA applied to excised tissues (cf. 5, 16), and such studies demonstrated that IAA was oxidatively decarboxylated to 3-methylenoxindole, 3-hydroxymethylxindole, or indole-3-aldehyde. Recently, Waldrum and Davies (20) showed that much of the IAA decarboxylation in pea epicotyl segments occurred at the cut surface owing to the tissue's cell wall localized peroxidases. Thus, it is possible that the magnitude of the peroxidative route for IAA catabolism has been overemphasized owing to the localization of peroxidase in cell walls (cf. 20) and to the use of in vitro peroxidase assays. In our studies (3, 13), the IAA was applied to the cut endosperm surface of the germinating seedling where cut surfaces of living cells are minimal; and it is possible that this more natural route of application via nutritive tissue reveals the in vivo pathway of IAA catabolism.

In this work, we report that OxIAA is an endogenous compound in shoot and endosperm tissue of Z. mays. The levels of OxIAA observed are similar to those of free IAA in these tissues. A previous abstract of these studies has appeared (14).

MATERIALS AND METHODS

Plant Tissue. Corn kernels, Zea mays cv Stowell's Evergreen Sweet Corn (W. Alee Burpee Co.), were surface sterilized in 1% NaOCl for 10 min, then soaked in running water at 25°C for 16 h. After imbibition, the seeds were grown in moist paper towels at 25°C and 80% RH (3) for an additional 8 h for the endosperm experiments, or were planted in trays of moist vermiculite for an additional 104 h for the shoot experiments. Both the paper towel-grown seedlings and the vermiculite-grown seedlings were harvested at similar stages of growth, 21.2 ± 4.2 cm and 22.0 ± 3.9 cm, respectively. A phototropically inactive green safe light was used during necessary manipulations.

Synthesis of Labeled OxIAA. [1-14C]OxIAA (10 μCi/μmol) was synthesized by the method of Hinman and Bauman (7). Purification was with LH-20 lipophilic Sephadex chromatography using 2-propanol:H2O (1:1, v/v) for elution. The UV spectrum, LH-20 retention volume, and the HPLC retention time on a C18 Whatman Partisil 10 ODS column (25 x 0.46 cm) as the free acid and as the PFB ester were as previously reported for OxIAA which had been characterized by MS (13). The radiolabeled purity was estimated to be 95% as measured both by chromatography on a C18 HPLC eluted with ethanol:H2O (1:4, v/v) plus 1% acetic acid (v/v), and by TLC developed with chloroform:methanol:H2O (85:14:1, v/v/v).

Extraction of Tissue. After harvesting, the tissue was placed onto solid CO2 and then stored at −20°C until used for extraction. Shoot or endosperm tissues were homogenized in a 4-liter Waring Blender in sufficient acetone to make the final acetone:H2O concentration 7.3 (v/v). All assays were by an isotope dilution method, and typical experiments are as follows: 2.46 x 106 dpm of 10 μCi/μmol OxIAA was added to 394 g of harvested endosperm tissue, and 2.42 x 106 dpm of 10 μCi/μmol OxIAA was added to 2.2 kg of harvested shoot tissue. After homogenization, the acetone:H2O extract (7.3, v/v) was evaporated under reduced pressure to an aqueous solution. For the shoot extract, the aqueous phase was partitioned three times with chloroform, and the chloroform phase discarded. The aqueous phase was then acidified to pH 3 and partitioned with ethyl acetate. The ethyl acetate fraction was evaporated to near dryness and chromatographed on a 10-ml bed volume DEAE Sephadex column, washed with ethanol:H2O (1:1, v/v) and eluted with ethanol:H2O (1:1, v/v) with a gradient of 0 to 5% (v/v) acetic acid. Inasmuch as the purity of the samples varied between experiments, additional partitionings were sometimes required prior to the DEAE Sephadex chromatography. Tubes containing radioactivity, at the retention volume of OxIAA

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2 Abbreviations: OxIAA, oxindole-3-acetic acid; PFB, pentafluorobenzyl; FID, flame ionization detector.
from the DEAE Sephadex chromatography, were pooled and taken to near dryness. The samples were resuspended in 2-propanol:H2O (1:1, v/v) and chromatographed on LH-20 lipophilic Sephadex and eluted with 2-propanol:H2O (1:1, v/v).

OxIAA from endosperm tissue was isolated as previously described (13). The acetone extract from the endosperm contains yellow lipidal material which was removed by partitioning of the OxIAA into n-butanol followed by chromatography with nonpolar solvents on DEAE cellulose. The samples were then chromatographed on a 5-ml bed volume DEAE Sephadex column followed by an LH-20 column as described for the shoot extracts. Both shoot and endosperm samples were then chromatographed on a 25 × 0.46 cm Partisil-10 ODS HPLC column and eluted with ethanol:H2O (1:4, v/v) containing 1% acetic acid (v/v). Radioactive fractions with the retention time of OxIAA were pooled and derivatized to the PFB ester (2). The PFB ester was further purified on C8 HPLC and eluted with ethanol:H2O (43:57, v/v).

GC for the Determination of Specific Radioactivity. The specific radioactivity of the reisolated OxIAA was determined by a modification of the double standard isotope dilution assay previously described for the measurement of IAA (1, 11). PFB-IAA of known specific radioactivity was used as the second internal standard in the determination of the specific radioactivity of OxIAA. All samples were chromatographed on a 1.8 m × 2 mm i.d., OV-17 column with the temperature at 200°C for 3 min followed by a temperature program of 200 to 250°C at 4°C·min⁻¹. The PFB-OxIAA and PFB-IAA were initially chromatographed separately to ensure the absence of interfering compounds. The PFB-IAA was purified prior to GC by C8 HPLC using ethanol:H2O (1:1, v/v) as eluant; however, GC indicated a contaminant in the PFB-OxIAA which would have interfered with the PFB-OxIAA quantitation. Reducing the ethanol concentration from 50 to 43% ethanol (v/v) resolved the PFB-IAA from the contaminant. The plant OxIAA sample contained no IAA detectable by GC (Fig. 1); and this was as expected, inasmuch as IAA was separated from OxIAA by LH-20 chromatography, and by HPLC steps. The PFB-IAA of known specific activity was then mixed with the plant PFB-OxIAA to give similar peak areas with a FID. The ratio of peak areas of PFB-IAA to PFB-OxIAA, and the ratio of radioactivity in the peaks of PFB-OxIAA to PFB-IAA were then measured. Peak areas were measured by cutting and weighing photocopies of the GC tracings, and radioactivities were measured by scintillation counting. The ratio of the area and the radioactivity in the peaks of the FID extinguished, and corrected for counting efficiency. From these measurements, the specific radioactivity of the reisolated OxIAA was calculated by means of the following equation:

\[ \frac{\text{IAA peak area \times OxIAA dpm \times specific activity}}{\text{OxIAA peak area \times IAA dpm \times of the IAA}} = \text{specific activity of the OxIAA} \]

The rationale of the double standard method for determination of specific activity has previously been described (1, 11). Briefly, however, the method utilizes a simple proportionality: if a compound of known specific radioactivity is coincided with a compound of unknown specific radioactivity, then the ratio of radioactivity per peak area of the known compound to that of the unknown may be used to calculate the specific radioactivity of the compound of interest.

Detector Sensitivity. The detector sensitivity of the FID for PFB-IAA and PFB-OxIAA was determined by separate injections of PFB-IAA and PFB-OxIAA of known specific activities. By measuring the peak area and radioactivity eluting from the column for each standard of known specific activity, the detector responses in peak area per amount of sample injected could be calculated as follows:

\[ \frac{1/\text{specific activity}}{1/\text{radioactivity or } l/\text{activity} \times \text{peak area} \times \text{detector sensitivity}} = \frac{\text{peak area/ng}}{l/\text{nCi}} \]

Because the FID essentially measures the mass of carbon in the compound, the detector sensitivity should be approximately the same for both IAA and OxIAA. The detector sensitivities observed were 5.1 area units/ng IAA as compared to 5.4 area units/ng OxIAA. The OxIAA values reported for endosperm and shoot tissues were not corrected for detector sensitivity since the sensitivity differences were within experimental error.

RESULTS

The amount of OxIAA present in the endosperm and shoot tissue was determined from the isotope dilution equation of Rittenberg and Foster (15):

\[ Y = [(C_i/C_0) - 1]X \]

The initial specific activity (C0) of OxIAA was known, the final diluted specific activity (Cf) of OxIAA was measured, and the amount of OxIAA (X) added to the tissue was known. With this information, the equation was solved for the endogenous amount of OxIAA in the tissue (Y).

OxIAA was estimated to be 900 and 927 nmol/kg fresh weight in the endosperm, and 220 and 414 nmol/kg fresh weight in the shoot (Table I) for average values of 914 and 317 nmol/kg fresh weight of OxIAA in endosperm and shoot tissue, respectively. The amount of OxIAA in endosperm tissue on a fresh weight basis was about 3 times higher than that in shoot tissue, while the amount of OxIAA per endosperm was about 7 times higher than the amount of OxIAA per shoot.

Epstein et al. (3) reported amounts of free IAA in dark-grown
OXINDOLE-3-ACETIC ACID IN CORN

Table I. A Quantitative Determination of the Amount of OxIAA in Z. mays Shoot and Endosperm Tissues by an Isotope Dilution Method

| Tissue          | Experiment No. | C' | C'' | X* | Y* | Tissue Fresh Wt | OxIAA
|----------------|----------------|----|-----|----|----|----------------|--------
| Endosperm      | 1              | 2.2 x 10^7 | 5.3 x 10^6 | 21.5 | 67 | 0.39 | 900 | 54
| Endosperm      | 2              | 2.2 x 10^7 | 5.2 x 10^6 | 21.5 | 69 | 0.39 | 927 | 362
| Shoot          | 1              | 2.2 x 10^7 | 4.1 x 10^6 | 21.1 | 92 | 2.2  | 220 | 44
| Shoot          | 2              | 1.6 x 10^7 | 2.2 x 10^6 | 12.6 | 79 | 1.0  | 414 | 54

* C', initial specific activity; C'', final specific activity; X, amount of OxIAA added to tissue; Y, endogenous amount of OxIAA.

b OxIAA per tissue was calculated from the OxIAA per fresh weight using the following conversion factors:

0.39 g fresh weight/endosperm in endosperm quantities 1 and 2, and 0.2 g fresh weight/shoot and 0.13 g fresh weight/shoot in shoot quantities 1 and 2, respectively.

Table II. Amount of IAA and OxIAA in Seedlings of Z. mays

<table>
<thead>
<tr>
<th>Tissue</th>
<th>OxIAA* Content</th>
<th>IAA* Content pmol/endosperm or shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosperm</td>
<td>357</td>
<td>308</td>
</tr>
<tr>
<td>Shoot</td>
<td>49</td>
<td>27</td>
</tr>
</tbody>
</table>

* Average values from Table I.

b IAA values from Epstein et al. (3).

DISCUSSION

In Z. mays endosperm (13) and in root and shoot segments (12), IAA is rapidly catabolized with retention of the carboxyl group. IAA catabolites which retain the carboxyl group, such as OxIAA, dioxindole-3-acetic acid, and their derivatives, have been reported in rice bran (9), Z. mays (10), Vicia faba (19), and Hygrophorus conicus (17). We demonstrated a precursor-product relationship between IAA and OxIAA in the endosperm of Z. mays (13) by showing the oxidation of [1-14C]IAA to [1-14C]OxIAA. The present work identifies OxIAA as a naturally occurring compound in both shoot and endosperm tissues. OxIAA occurs at levels similar to that of free IAA in these tissues with 49 pmol OxIAA/shoot and 27 pmol IAA/shoot, and 357 pmol OxIAA/kernel and 308 pmol IAA/kernel.

With the knowledge that (a) OxIAA is a naturally occurring compound in Z. mays shoot and endosperm tissues, (b) in vivo oxidation of IAA to OxIAA is a major pathway in endosperm tissue, and (c) OxIAA is generally inactive in growth promotion (6, 9, 18, 21) — although a contrary report has appeared (4), we postulate that the oxidation of IAA to OxIAA plays a role in the regulation of IAA concentrations and thus in the amount of IAA-mediated growth. Further, there are kinetic reasons why the hormone may be destroyed simultaneously with its action, or shortly after its action, and thus knowledge of the mechanism and site of oxidation of IAA to OxIAA may be important in attaining an improved understanding of how IAA regulates growth.

LITERATURE CITED
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