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Oxidation of Indole-3-Acetic Acid to Oxindole-3-Acetic Acid by an Enzyme Preparation from *Zea mays*[1]

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ABSTRACT

Indole-3-acetic acid is oxidized to oxindole-3-acetic acid by *Zea mays* tissue extracts. Shoot, root, and endosperm tissues have enzyme activities of 1 to 10 picomoles per hour per milligram protein. The enzyme is heat labile, is soluble, and requires oxygen for activity. Cofactors of mixed function oxygenase, peroxidase, and intermolecular dioxygenase are not stimulatory to enzymic activity. A heat-stable, detergent-extractable component from corn enhances enzyme activity 6- to 10-fold. This is the first demonstration of the *in vitro* enzymic oxidation of indole-3-acetic acid to oxindole-3-acetic acid in higher plants.

OxIAA[2] is a naturally occurring catabolite of IAA in *Zea mays* (15). The peroxidative decarboxylation pathway is a minor component in corn since feeding [1 – ^{14}C]IAA to either endosperm of entire seedlings, or root or shoot pieces results in only 1 to 5% decarboxylation per hour of the labeled IAA (2, 14). Twenty-four h incubations of *Zea mays* tissues with labeled IAA result in the synthesis of OxIAA, and the further oxidation of OxIAA to 7-OH-OxIAA and 7-OH-OxIAA-Glc (13, 10). Seven-OH-OxIAA and 7-OH-OxIAA-Glc are also naturally occurring compounds in corn (10, 13).

Recently, Tsurumi and Wada (19, 20) have shown that IAA is conjugated to IAA-aspartate and oxidized to a conjugate of DiOxIAA (3-[O-β-glucosyl]-2-indolone-3-acetylaspartic acid) in the dicot *Vicia faba* (19, 20). DiOxIAA is oxidized at the 2 and 3 positions of the indole ring while OxIAA is oxidized at only the 2 position of the ring, both with carboxyl retention. OxIAA and DiOxIAA and their 5-hydroxy analogs were also shown to occur naturally in *Oryza sativa* (rice) bran (8). The occurrence of IAA catabolites which retain their carboxyl moiety in monocots and a dicot may indicate a wider distribution of nondecarboxylating pathways.

The *in vitro* decarboxylation of IAA catalyzed by horse radish peroxidase is well characterized (11). The present work is the first demonstration of an enzyme system which catalyzes oxidation of IAA without decarboxylation. A previous abstract of these studies has appeared (17).

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[2] Abbreviations: OxIAA, oxindole-3-acetic acid; DiOxIAA, dioxindole-3-acetic acid; 7-OH-OxIAA-Glc, 7-hydroxy-oxindole-3-acetic acid 7′-O-β-D-glucopyranoside; 7-OH-OxIAA, 7-hydroxy-oxindole-3-acetic acid; PFB, pentafluorobenzyl.

MATERIALS AND METHODS

Plant Materials. *Zea mays* cv Stowell's Evergreen Sweet corn kernels (W. Atlee Burpee Co.) were surface sterilized in 1% NaOCl for 10 min, then soaked in running water at 15°C for 16 h. After imbibition, the kernels were grown in moist paper towels for an additional 80 h at 25°C and 80% RH. Shoot (coleoptile plus primary leaves and mesocotyl), kernel (endosperm plus scutellum), and root tissues were excised and harvested into ice-chilled beakers, using a phototropically inactive green light for manipulations. For the sterile-culture experiments, kernels were germinated on sterilized moist paper towels in 2.5 × 25 cm culture tubes.

Enzyme Preparation. Endosperm, shoot, or root tissues were homogenized in 0.05 M phosphate buffer (pH 7.0) containing 5% (w/w tissue) insoluble polyvinylpyrrolidone with a tissue to buffer ratio of 2:3 (w/v). Triton X-100 0.4% (v/v) was added to the buffer extraction medium where indicated. Following homogenization of plant tissue, the buffer extract was filtered through two layers of cheesecloth and centrifuged at 12,000*g* for 10 min. The resulting supernatant fluid was dialyzed overnight against 2.5 L of extraction medium. The dialyzed solution was centrifuged at 12,000*g* for 10 min; the supernatant fluid was assayed immediately for activity or frozen in liquid N_2 for subsequent assays. Over 80% of the initial enzymic activity could be recovered following 2 months of storage at −196°C.

Enzyme Assay. The standard assay included 0.9 ml of enzyme plus 0.1 ml of addendum (metal ions, sulfhydryl reagents, or cofactors of oxidation reactions dissolved in 0.05 M phosphate buffer, see "Results"). [1 – ^{14}C]IAA (57 mCi · mmol^{-1}, 50 μCi · ml^{-1} Amersham) diluted with 2-propanol 2:8 v/v was the substrate for the assay. The radiolabeled IAA was 98% radiochemically pure as determined by C18 HPLC (16) with ethanol:H_2O:acetic acid (20:79:1 v/v/v, solvent system I) as eluant. The enzymic reaction was initiated by the addition of 10 μl of the diluted [1 – ^{14}C]IAA (1.7 nmol) to the enzymic mixture, and incubated with shaking (Dubnoff Metabolic Shaking Incubator, Precision Scientific Co.) at 30°C for 1 to 12 h. The reaction was terminated by the addition of acetone to the assay mixture 2:1, v/v. Acid precipitation of the protein was avoided to reduce nonenzymic breakdown of the acid-labile IAA and OxIAA. Carbon-14 recoveries for the enzyme reaction were determined by mixing a 20 μl aliquot of the enzyme mixture with 200 μl of 0.1% aqueous phosphoric acid, followed by the addition of scintillation cocktail (Safety-solve RPI). Radioactivity was measured on a Beckman LS 7000 scintillation counter. Samples were frozen at −20°C until assayed by HPLC. Samples were centrifuged at 12,000*g* for 10 min; the resulting supernatant fluid was evaporated to near dryness with reduced pressure at 35°C. Ethanol, H_2O, and concentrated acetic acid were added to the sample to make a final concentration of 20:79:1 (v/v/v), respectively, in 200 μl. A 10 μl aliquot was mixed with scintillation cocktail, and the radioactivity was counted to estimate recovery.

The remainder of the sample was chromatographed on a Varian 5000 HPLC, 0.46 × 25 cm 10 μm Partisil 10 ODS column (Whatman, Inc.) with a Co:Pel ODS precolumn (Whatman, Inc.), and eluted with solvent system I at 1 ml/min. Eluant between 5 and 8.5 min was collected in 0.5 ml fractions and radioactivity determined by scintillation counting. OxIAA had a retention time of 7.5 to 7.8 min under these conditions, and 7-OH-OxIAA and IAA had retention times of 5.6 and 18 min, respectively. For more complete radioactive profiles, eluant between 2 and 20 min was collected. In later experiments, the solvent system was switched at 8 min to 100% ethanol and 2 ml/min resulting in elution of IAA at 14 min. This method decreased the assay time (baseline resolution between IAA and OxIAA was maintained) and cleaned the column between injections. A channel ratio H# method (Beckman ^{137}Ce quench monitoring) showed the efficiency of radioactivity counting to be 82 to 85%.

The amount of [^{14}C]OxIAA formed was corrected for recovery using [^{14}C] recovery, and converted into nmol of OxIAA by multiplication by the specific radioactivity:

$$[^{14}C]OxIAA_{HPLC} \times \frac{[^{14}C]_{INITIAL}}{[^{14}C]_{HPLC}} \times \frac{\text{initial nmol IAA}}{[^{14}C]IAA_{INITIAL}} = \text{nmol OxIAA synthesized}$$

The equation is valid since the specific radioactivity of IAA and the OxIAA synthesized are the same, and since the recoveries of IAA and OxIAA are essentially identical following HPLC. Since $[^{14}C]_{INITIAL} = [^{14}C]IAA_{INITIAL}$, the equation can be reduced to:

$$\frac{[^{14}C]OxIAA_{HPLC}}{[^{14}C]_{HPLC}/[^{14}C]\text{reaction recovery}} \times \text{initial nmol IAA} = \text{nmol OxIAA synthesized}$$

where $[^{14}C]OxIAA_{HPLC}$ = [^{14}C]OxIAA isolated following HPLC; $[^{14}C]_{HPLC}$ = total radiolabel applied to HPLC; [^{14}C] reaction recovery = % recovery of radiolabel at termination of the enzymic reaction (this factor corrects for underestimation of [^{14}C] recovery due to enzymic decarboxylation); initial nmol IAA = nmol IAA added to the enzyme assay; and $[^{14}C]IAA_{INITIAL}$ and $[^{14}C]_{INITIAL}$ = radiolabeled IAA added to the enzyme assay.

Validation of Enzyme Assay. The enzyme assay was validated by a reverse isotope dilution assay (4). Unlabeled OxIAA was added to the enzyme mixture following termination of the reaction by acetone. The specific radioactivity of the OxIAA was determined by measuring the radioactivity in the OxIAA HPLC peak *versus* the area of the 254 nm absorbance of the peak. The 254 nm absorbance area was integrated by an IBM 9000 computer and compared to an OxIAA standard curve (absorbance *versus* OxIAA amount). Results from the two quantitation methods were identical, and the radioactivity recovery method (see preceding section) was routinely used to quantitatively estimate OxIAA synthesized.

To determine whether 7-OH-OxIAA was synthesized by the *in vitro* system the following solvent system II (10) was used: 10% A plus 90% B from 5 to 20 min (A = ethanol plus 0.1% acetic acid, and B = H_2O plus 1% acetic acid).

Biological activity of OxIAA was measured by a *Z. mays* mesocotyl straight growth assay according to the method of Nitsch (12). Mesocotyl sections (4.6 ± 0.1 mm cut 2 mm below the coleoptile node) were incubated in the dark for 20 h in pH 5.0 citrate-phosphate buffer plus 20 mg/L of chloramphenicol, 2% sucrose (w/v), and IAA or OxIAA. Mesocotyl length was measured to the nearest 0.1 mm using a dissecting microscope.

Gas chromatography of the putative pentafluorobenzyl ester of OxIAA was performed on a Varian 3700 gas chromatograph with a 2 mm i.d. × 2 m 3% OV-17 (Gas chrom Q, Applied Science) column using N_2 (30 ml/min) as the carrier gas. The PFB-OxIAA was chromatographed at 220°C isothermally, and detected by FID. The acid ring expanded OxIAA (2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid) (7) was chromatographed on C18 HPLC and eluted with solvent system I. Methyl-2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid and PFB-OxIAA were eluted from C18 HPLC by ethanol:H_2O 30:70 (v/v) and 43:57 (v/v), respectively.

The oxygen requirement for the reaction was examined by 10 min vacuum evacuation of 10 ml Thunberg tubes containing the complete reaction mixture, followed by a 30 second argon or air flushing. The evacuation and flushing were repeated 3 times at 0°C. The Thunberg tubes were incubated at 30°C for 4 h and the reaction mixture assayed for OxIAA formation as previously described.

The sedimentation characteristics of the enzyme were examined by centrifugation at 100,000 *g* for 1 h on a Spinco model L centrifuge with a 50Ti rotor. The preparation was assayed prior to centrifugation, and the supernatant fluid and resuspended pellet were assayed following centrifugation.

RESULTS

Since the enzymic assay is an end-point analysis the linearity over time and protein concentration was determined. The enzymic reaction was a linear function of time up to 4 h, and slowed between 4 to 6 h. The leveling off of activity could not be accounted for by the depletion of substrate IAA. Enzymic activity for endosperm plus scutellum was linear over the protein concentration range 0.1 to 2.4 mg protein · ml^{-1}, and linear from 0.1 to 1 mg protein · ml^{-1} for vegetative mesocotyl tissue. Standard incubation periods were 4 h for detergent extracted enzyme and buffer extracted enzyme, with 1 to 2 mg protein · ml^{-1}. The pH optimum for the enzyme assay was 7.0. Enzymic activity was destroyed after 5 to 10 min at 100°C.

The enzymic activity from sterilely cultured corn seedlings and nonaseptically cultured corn was 1.1 pmol · h^{-1} · mg^{-1} protein and 1.0 pmol · h^{-1} · mg^{-1} protein, respectively. Fungal contamination was not present in the aseptically grown corn as shown by culturing of corn and media from the aseptically cultured corn on potato dextrose agar. Thus, the oxidation rate of IAA by nonaseptically grown corn was not influenced by microbial contamination, and corn kernels sterilized with 1% NaOCl and grown nonsterilely were used routinely for enzyme preparations.

Triton prepared enzyme from shoot, root, and endosperm tissues had 1 to 10 pmol · h^{-1} · mg^{-1} protein of enzymic activity. The highest activity was from endosperm tissue at 6 to 10 pmol · h^{-1} · mg^{-1} protein, while shoot and root tissues had 3 to 5 pmol · h^{-1} · mg^{-1} protein. Vegetative tissues had the lowest recovery of radioactive ^{14}C following the assay with 30 to 60% recovery. For vegetative tissues, the lower rate of oxidation of IAA to OxIAA may be partly explained by the lower availability of substrate IAA due to a competing peroxidase decarboxylation reaction. Peroxidase activity has been reported for corn tissues, but its *in vivo* role in oxidizing IAA has been shown to be minimal (2, 14).

The chromatographic properties of the product of IAA's enzymic oxidation are shown in Table I. Chromatography as both the free acid, pentafluorobenzyl ester, and acid ring-expanded OxIAA were similar for both authentic and enzymically synthesized OxIAA. OxIAA has been shown to be a naturally occurring compound by GC-MS in *Z. mays* tissues (16) and chromatographic evidence also supports the *in vitro* oxidation of IAA to OxIAA. The further oxidation of OxIAA to 7-OH-OxIAA or 7-OH-OxIAA-glc was not observed in the *in vitro* system. A polar catabolite of IAA with a similar retention time to 7-OH-OxIAA with HPLC solvent system I was often synthesized along with OxIAA, but it was separated from 7-OH-OxIAA under C18 HPLC solvent system II (see "Materials and Methods"). The unknown had a retention volume of 20 to 21 ml and the

Table I. *Authentic OxIAA and Enzymically Synthesized Putative OxIAA Have the Same Chromatographic Properties as Evidenced by HPLC and GLC*

Chromatography		Retention Time	
		OxIAA	Enzyme product
		min	
HPLC	Free acid	7.5	7.5
HPLC	PFB ester	11.8	12.0
GLC	PFB ester	17.3	17.5
HPLC	Quinoline	6.5	6.5
HPLC	Quinoline methyl ester	6.1	6.0

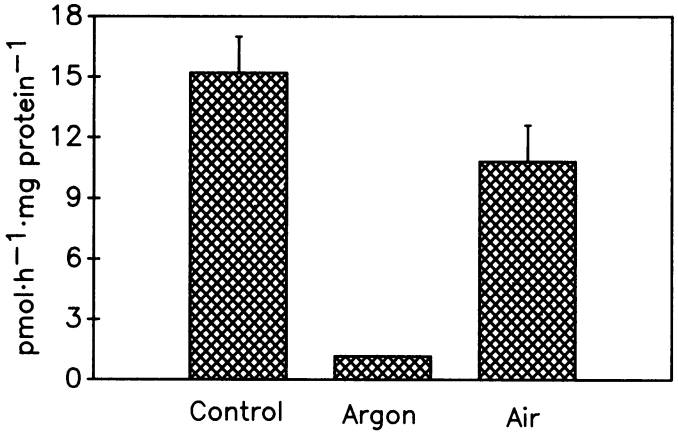

FIG. 1. Enzymic activity following evacuation and gas flushing of Thunberg tubes. Ultrapure Ar (99.995%, Matheson) was used in these experiments. Preliminary experiments with 99.9% N_2 showed inhibition of activity up to fourfold. Control refers to the enzymic activity prior to gas evacuation and flushing.

Table II. *Sedimentation Characteristics of Shoot and Endosperm Enzyme Preparations*

Treatment	Endosperm	Shoot
	pmol·h⁻¹	
Homogenate	44	25
100,000 *g* pellet	6.9	4.1
100,000 *g* supernatant	47	35

7-OH-OxIAA 24 ml with HPLC solvent system II; the putative and authentic OxIAA had retention volumes of 30 to 31 ml.

Flushing Thunberg tubes three times with Ar decreased enzymic activity nine times *versus* the air flushed control (Fig. 1) showing oxygen was required for optimal enzymic activity. However, demonstration of oxygen incorporation into the indole ring awaits $^{18}O_2$ experiments.

When enzyme preparations were centrifuged at 100,000*g* for 1 h, both shoot and endosperm preparations retained most of the activity in the supernatant fluid, 90 and 87%, respectively (Table II). Minor activity remained in the unwashed pellet. Sedimentation characteristics of the enzyme preparation resembled a soluble enzyme and not a microsomal enzyme. The results were similar whether the enzyme was prepared with or without Triton X-100.

Inorganic ions and cofactors of oxygenase reactions were tested for enhancement of enzymatic activity (Tables III and IV). Ca^{2+} and Fe^{2+} were not stimulatory at 50 μM concentrations, while Zn^{2+} at 5 μM was inhibitory by 15 to 20%. Mercaptoethanol and dithiothreitol were inhibitory to enzymic activity by 80 to 100% when added at 5 to 50 μM. Cofactors of peroxidase, Mn^{2+}, H_2O_2, and 2,4-dichlorophenol, decreased ^{14}C recovery from the enzyme reaction by over 50%, without increasing the amount of OxIAA synthesized. Peroxidase plus Mn^{+} and H_2O_2 decarboxylates IAA to hydroxymethyloxindole, indole-3-aldehyde, etc. but does not decarboxylate OxIAA (6). The oxidation of IAA to OxIAA is thus a separate pathway from the well studied *in vitro* oxidative decarboxylation of IAA by peroxidase.

Since one oxygen atom is added to the IAA molecule to form oxindole-3-acetic acid, a mixed function oxygenase reaction was indicated. For such a reaction, a reductant would be required since one oxygen would be incorporated into the indole ring and the other oxygen reduced to water. However, NADPH at 0.67 or at 1.2 mM was not stimulatory to the enzyme's activity. NADPH plus pterin (6,7 dimethyl-5,6,7,8 tetra-hydropterine, Sigma), 1.2 and 0.5 mM, respectively, were also not active. At low concentrations (10 μM), flavin adenine dinucleotide was not stimulatory to enzymic activity.

Fe^{2+},α-ketoglutarate, and ascorbic acid are co-substrates for the oxidation of gibberellins (5) by an intermolecular dioxygenase reaction by co-oxidation of α-ketoglutarate to succinate and CO_2. But oxidation of IAA to OxIAA was not increased by Fe^{2+}, α-ketoglutarate, ascorbate at 1, 1, and 5 mM concentrations, respectively. The substitution of NADPH for ascorbate was also not stimulatory. Thus, none of the most likely types of oxidation reactions, peroxidase, mixed function oxygenase, or intermolecular dioxygenase were involved in the oxidation of IAA to OxIAA since cofactors and co-substrates of these reactions were ineffective.

To increase recovery of the enzymic activity during extraction, the nonionic detergent Triton X-100 was added to the homogenization medium. Following preparation with and without 0.4% Triton X-100, the enzyme was dialyzed overnight against 0.05 M phosphate buffer, and then assayed. Triton X-100 addition to the homogenate prior to the enzyme assay had no effect on enzymic activity, but homogenization of tissue with buffer plus Triton X-100 enhanced enzymic activity by up to 10-fold. If Triton X-100 was added to the homogenate prior to dialysis (absent from the tissue homogenization medium), activity was increased twofold without an increase in protein recovery. These experiments indicated that the Triton effect could be due to the preferential extraction of a lipid-soluble enzyme, or of the extraction of a lipid soluble cofactor or co-substrate. The total amount of protein recovered by buffer with and without Triton X-100 was similar, so the possible presence of a cofactor was studied by adding boiled Triton prepared enzyme to enzyme (prepared without Triton X-100). The addition of the boiled Triton extract increased enzyme activity up to sixfold (Fig. 2). The addition of boiled enzyme (without Triton X-100 preparation) was ineffective in stimulating enzymic activity. These results indicated that a heat-stable, lipid-soluble factor was extracted by Triton X-100 and was responsible for the detergent enhancement of enzymic activity.

OxIAA has been previously shown to be inactive in stimulating plant growth in five bioassays (*cf.* 5), although the initial report for pea stem sections showed stimulation. Solutions of 10^{-7} to 10^{-3} M OxIAA and IAA were assayed in a corn mesocotyl bioassay. OxIAA was inactive from 10^{-7} to 10^{-4} M, while IAA was stimulatory over the same concentration range with maximum activity at 10^{-7} M (Table V). These results support the view that the oxidation of IAA to OxIAA results in loss of biological activity for the molecule.

DISCUSSION

An enzyme system which will oxidize IAA at the 2 position of the indole ring while retaining the carboxyl side chain has been partially characterized in enzyme preparations of corn. This

Table III. *Effect of Inorganic Ions, and Sulfhydryl Reagents on the Enzymic Oxidation of IAA to OxIAA with Endosperm Enzyme Preparations and Triton X-100 Endosperm Enzyme Preparations*

Treatment	Concentration	Endosperm (Triton)	Concentration	Endosperm
	μM	% of control[a]	μM	% of control[b]
Fe^{2+}	200	109	500	112
Ca^{2+}	50	100	25	72
Cu^{+}	ND[c]		20	101
Zn^{2+}	5	79	ND	
Mn^{2+}	50	81	100	90[d]
Mercaptoethanol	5000	1	5000	0
Dithiothreitol	ND		5000	0

[a] Control value, 8.3 ± 1.8 pmol · h^{-1} · mg^{-1} protein. [b] Control value, 3.8 ± 1.1 pmol · h^{-1} · mg^{-1} protein. [c] Not determined. [d] Plus 17 mM H_2O_2.

Table IV. *Effect of Cofactors and Co-substrates of Oxygenases and Peroxidase Reactions on the Oxidation of IAA to OxIAA by Endosperm Enzyme Preparations*

Treatment	Tissue
	endosperm (Triton X100) % of control[a]
2,4 dichlorophenol 5 μM	87
2,4 dichlorophenol 5 μM + Mn^{+} 5 μM	97
Fe^{2+} 50 mM + 500 μM Pterin	93
	endosperm % of control[b]
Pterin 500 μM + 1.2 mM NADPH	42
FAD 10 μM + 1.2 mM NADPH	38
Fe^{2+} 1 mM + 1.2 mM NADPH + α-keto-glutarate 1 mM	21
Fe^{2+} 1 mM + 5.7 mM ascorbate + α-keto-glutarate 1 mM	0
Mn^{2+} 0.1 mM + 17 mM H_2O_2	90
NADPH 0.7	90

[a] Control value, 8.3 ± 1.8 pmol · h^{-1} · mg^{-1} protein. [b] Control value, 3.8 ± 1.1 pmol · h^{-1} · mg^{-1} protein.

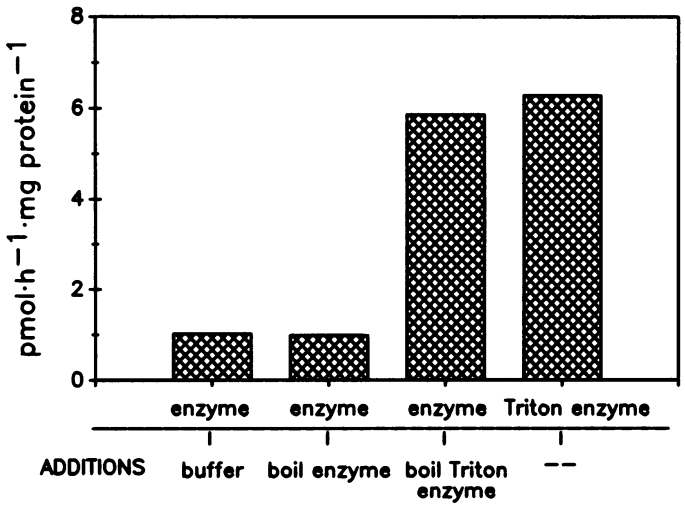

FIG. 2. Stimulation of enzymic activity by Triton X-100 preparation of enzyme, and by the addition of the Triton X-100 heat-stable factor to enzyme prepared without detergent.

Table V. *Biological Activity of IAA and OxIAA in the Enhancement of Dark-Grown Corn Mesocotyls*

Eleven to 12 sections were assayed in each treatment, and data is expressed as treatment length minus initial length. IAA at 10^{-7} to 10^{-4} M caused a significant increase in length at the 0.01 level using Duncan's Multiple range test to determine mean separation.

Concentration	IAA	OxIAA
M	mM	
0	1.4 ± 0.4	1.4 ± 0.4
10^{-7}	3.0 ± 1.1	1.4 ± 0.3
10^{-6}	2.6 ± 0.7	1.6 ± 0.6
10^{-5}	2.4 ± 0.8	1.5 ± 0.3
10^{-4}	2.4 ± 0.6	1.5 ± 0.4
10^{-3}	1.8 ± 0.4	1.9 ± 0.4

is a novel enzyme system discrete from the peroxidative oxidation of IAA since peroxidase cofactors do not stimulate formation of OxIAA. OxIAA was shown not to be an intermediate in horse radish peroxidase's decarboxylation of IAA (6).

The reaction is not a typical mixed function oxygenase reaction in that the enzyme is soluble, and is not stimulated by cofactors of mixed function oxygenases. The intermolecular dioxygenases are soluble enzymes as in the oxidation of GA_{29} aldehyde dioxygenase (5), but cofactors of this reaction do not stimulate IAA oxidation.

The enzyme requires oxygen for optimum activity. In oxidation of IAA by peroxidase to 3-methyleneoxindole, $^{18}O_2$ and $H_2{}^{18}O$ experiments showed that H_2O is the source of oxygen in the oxindole ring (11). The source of the oxygen in the oxindole nucleus of oxindole-3-acetic acid remains to be identified by heavy isotope experiments.

The reaction's stimulation by Triton X-100 preparation of the enzyme, or by the addition of boiled Triton X-100 prepared enzyme indicates involvement of a heat-stable, lipid-soluble component in the reaction. The existence of a lipid-soluble, heat-stable component is currently being investigated for this novel reaction.

The extent of peroxidase's involvement in catabolism of IAA needs to be reinvestigated. In corn, decarboxylation of IAA was only 5 to 12% of the turnover for intact tissues, while isolated corn peroxidase readily catalyzed the oxidation of IAA to seven major decarboxylated products (1).

In pea, peroxidase activity was mainly a cut surface phenomenon which could largely be washed away, and was proportional to the number of pieces into which the tissue was cut (21). In

Pinus sylvestris, Scots Pine, an *in vitro* system rapidly metabolized IAA to indole-3-methanol and four other decarboxylated metabolites. However, when IAA was fed to Scots pine protoplasts, IAA was more slowly metabolized to two compounds: an unidentified carboxyl-retaining catabolite and indole-3-methanol (minor catabolite) (18). Recently, isotope dilution experiments have confirmed that oxindole-3-acetic acid is a naturally occurring compound in *Pinus sylvestris* seedlings (3). The nondecarboxylation pathways observed in the dicots *Vicia faba* and *Pinus sylvestris*, and the monocot *Z. mays* (implicated in *Oryza sativa*, *Ribes rubrum*, and *Brassica rapa*, [8, 9]) warrant the reexamination of the route(s) of IAA catabolism in plants, and suggest that the decarboxylation pathway may have been overestimated by the experimental conditions utilized.

In corn, IAA is oxidized to OxIAA; OxIAA may be further oxidized to 7-OH-OxIAA and 7-OH-OxIAA-glc. The first enzyme in this pathway has been partially characterized. Since catabolic oxidation of IAA to OxIAA is the first reaction in the catabolic pathway, and is apparently irreversible (it is inactive in bioassays including corn) it may have an important role in regulating the steady state level of IAA during IAA-mediated growth.

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