Influence of Ethylene on Indole-3-acetic Acid Concentration in Etiolated Pea Epicotyl Tissue

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ABSTRACT

Indole-3-acetic acid levels are diminished about 50% in 5- to 6-day-old epicotyls of etiolated pea (Pisum sativum L.) seedlings treated with 10 to 36 μl/l ethylene for 18 to 24 hr.

Supraphysiological concentrations of auxins (above 10 μM) induce ethylene biosynthesis in etiolated pea seedlings and other plant tissue systems (10, 13). The reverse physiological effect, that is, the lowering of auxin levels by ethylene, has also been reported (12, 14-16). On the other hand, some studies suggest an increase in auxin levels in tissues exposed to ethylene (7). The supporting evidence for both auxin responses to ethylene is based on auxin bioassay techniques. An assay specific for auxin could clarify these contradictions in the literature. In this report the direct chemical analysis of indole-3-acetic acid was carried out on purified extracts of etiolated pea seedlings grown in the presence and absence of ethylene. We find that concentrations of IAA are about half those of controls in 5-day-old etiolated pea seedlings grown for about 1 day in ethylene at concentrations which produce the marked subhook thickening.

MATERIALS AND METHODS

Alaska pea seeds were surface-sterilized and germinated, as previously described, in 12-l desiccators (10). When seedlings were 4 days old, the desiccators were sealed. Ethylene was introduced into one-half of the desiccators so that the final concentration was 10 to 20 μl/l, and the seedlings were allowed to grow an additional 24 hr. The seedlings were removed at the end of the 5th day, counted into groups of 200, weighed and lyophilized. Epicotyls were removed from the lyophilized seedlings by razor blade for IAA extractions, and the cotyledons and roots were discarded. The procedure for extraction and purification of IAA conducted under N2 (bag) and dim light was essentially that of Mann and Jaworski (11) with some modification. The dried epicotyls were ground in a mortar to a powder, and about 20 g of dry, powdered epicotyls was extracted with 500 ml cold 95% ethanol containing 0.1 mM DIECA.2 The extract was filtered, and the residue was again extracted with 500 ml of the ethanol-DIECA solution. [2-14C]IAA (1 μCi, 7.9 mCi/mM) was added to the extract to enable check for losses in subsequent extraction and purification procedures. The recovery of labeled IAA was on average 47%

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2 Abbreviations: DIECA: diethylthiocarbamate; TFA: trifluoracetic acid.

for controls and 51% for ethylene-treated tissue. The extract was reduced to an aqueous solution on the rotary evaporator, made to pH 9 with 1 N NaOH, and allowed to stand 20 min for hydrolysis of IAA esters. The aqueous extract was then acidified with cold 6 N HCl to pH 2.5 and extracted with three 200-ml portions of peroxide-free ether. Water was removed from combined ether extracts with sodium sulfate. The antioxidant tolulhydroquinone was added to the filtered (sintered glass) ether extract, which was then evaporated to dryness in a rotary evaporator. The vacuum was broken with N2, and the residue was taken up in a few ml of 1:1 methanol-chloroform. This residue was purified by passage through 20 g of acid-washed activated alumina (Brockman 1). IAA was eluted from the column with about 50 ml methanol-ammonium hydroxide-H2O (7:2:1). A small amount of sodium metabisulfite was added to the eluate, and the excess ammonia and methanol were removed from an evaporating dish with a gentle stream of N2. The aqueous residue was acidified with 2.5 N HCl to pH 2.5 and extracted with four 15-ml portions of peroxide-free ether. The combined ether extracts were dried over sodium sulfate and evaporated to near dryness in a N2 stream. The concentrated ether extract was streaked on Whatman 3MM chromatography paper that had been washed with both HCl-H2O (1:1) and methanol-H2O (1:1). The chromatogram was developed by ascending elution with 5% acetic acid. The IAA was located on the paper by chromatography with authentic IAA, which was identified by fluorescence under UV after reaction with acetic anhydride-TFA (14). The IAA band was cut off and eluted with four 100-ml portions of methanol. The eluate was filtered through sintered glass and evaporated to dryness on the rotary evaporator to near dryness. The IAA residue was transferred to a small vial with a minimum amount of methanol. This residue from paper chromatography was streaked onto a silica gel TLC sheet previously washed with redistilled methanol. The TLC sheet was placed in a "sandwich" apparatus, and the chromatogram developed with a freshly prepared solution of isopropyl ether-acetic acid (95:5). IAA was located as described above. The band was scraped into a centrifuge tube containing redistilled methanol for elution. IAA was assayed by formation of the fluorescent IAA derivative with acetic anhydride and TFA according to Stoessl and Venis (14). Quenching in the extract was accounted for by use of proper quench curves prepared with sample and IAA standards. Fluorescence was determined with an Aminco-Bowman spectrofluorometer; the excitation wavelength was 440 nm, and fluorescence emission was measured at 490 nm. The data in these experiments with 'Alaska' pea seedlings represent eight separate experiments in which a total of 2,980 control seedlings and 6,620 C2H2-treated seedlings were extracted.

A similar series of experiments were carried out with pea seedlings of another variety, 'Sweet Eminent,' by an improved extraction and assay technique for IAA determination (8).
‘Sweet Eminent’ seedlings were grown for 4 days in open desiccators and then sealed in \( \text{C}_2\text{H}_4 \) atmospheres of about 19 to 36 \( \mu \text{l} \) for 18 to 22 hr before removal for assay. Control seedlings were similarly subjected to sealed atmospheres of normal air. Epicotyl tissue was rapidly weighed out in 8- to 11-g units, and quickly frozen and powered in liquid N\(_2\). The extraction, purification, and assay procedure was as previously described (8). This technique has the advantage of shortening the time required for a determination from days to a few hr and reduces losses. Addition of a known amount of radioactive IAA to the extract allowed correction for losses. Recovery of labeled IAA was on average 39\% for controls and 34\% for ethylene-treated tissue. In this procedure special care was taken to redistill all solvents and reagents so that quenching and extraneous fluorescence would be eliminated or reduced. Four separate experiments were carried out with 482 control seedlings and 617 \( \text{C}_2\text{H}_4 \)-treated seedlings.

RESULTS

With both ‘Alaska’ and ‘Sweet Eminent’ seedlings, the epicotyls treated with \( \text{C}_2\text{H}_4 \) contained less than 50\% the amount of IAA in controls (Table I). On a per gram fresh weight basis the differences were not as marked since the control seedlings on average weighed about 70 and 30\% more, respectively, for ‘Alaska’ and ‘Sweet Eminent’ than ethylene-treated seedlings. Although there was a large difference in IAA levels between ‘Alaska’ and ‘Sweet Eminent’ seedlings the percentage differences between treated and untreated seedlings were of the same order, about 50\%. The much higher level of IAA found in the ‘Alaska’ seedling is probably related to the extraction technique. Unlike the extraction procedure for ‘Sweet Eminent’ seedlings, extraction of Alaska seedlings included a step in which 1 n NaOH was used for hydrolysis of the IAA esters. Ueda and Bandurski (18) have shown that most of the IAA in maize seeds is bound in various esters and may be released by alkaline hydrolysis. Aside from the differences in the levels of IAA, epicotyls of ethylene-treated seedlings clearly contained less than half of the IAA present in control seedlings.

DISCUSSION

Our data show that \( \text{C}_2\text{H}_4 \) treatment of 24 hr or less diminished IAA content in the epicotyls of etiolated pea seedlings to about 50\% that of controls. These results agree with those of a number of studies (2, 4, 12, 15-17) in which untreated and ethylene-treated plant tissues were compared with respect to their levels of diffusible or extracted auxins bioassayed by the Avena coleoptile methods. Assays of auxin in our experiments were specific for IAA and therefore provide greater certainty on this matter.

In a few studies ethylene was found not to affect auxin levels (7). In some of those studies, the finding was likely due to crude methodology (use of illuminating gas) or variations in distribution of auxin in different tissues. Burg et al. (2) found increases in diffusible auxin in leaf and apex tissue of 7-day-old pea seedlings exposed to 10 \( \mu \text{l} \) \( \text{C}_2\text{H}_4 \) for 24 hr. These seedlings contained reduced auxin levels in the epicotyl hook and markedly reduced auxin levels in the subapical region. Other factors to consider are the length of exposure time to ethylene and the time interval between ethylene treatment and extraction or diffusion of auxin from the tissues. Also, it is possible that inhibitory substances included with the IAA extracts or diffuses obscured real differences.

Two questions relate to the diminished auxin content in the ethylene-treated tissues. One concerns the mechanism by which ethylene reduces auxin content of tissues and the other concerns the significance of this reduced auxin level. The effect of ethylene on polar transport may influence diffusible auxin (1, 3) but it has no effect on extractable auxin, which is the diminished auxin in the ethylene-treated seedlings that was determined in our experiments. Consequently, auxin binding, degradation, or synthesis must have been involved in the \( \text{C}_2\text{H}_4 \) action. Goren et al. (5) reported that \( \text{C}_2\text{H}_4 \) does not induce indole-acetylaspartate formation; this suggests that \( \text{C}_2\text{H}_4 \) does not alter conjugation of IAA. Our experiments showed that the percentage reduction of auxin levels in ethylene-treated ‘Alaska’ and ‘Sweet Eminent’ epicotyls was the same whether or not the extracts were hydrolyzed with alkali. Therefore, altering ester formation in the presence of ethylene appears not to have been the mechanism by which \( \text{C}_2\text{H}_4 \) diminished concentrations of IAA in pea epicotyls. The possibility that \( \text{C}_2\text{H}_4 \) causes destruction of auxin has been suggested (6, 12). This possibility needs to be more thoroughly explored. There is also a suggestion that \( \text{C}_2\text{H}_4 \) diminishes auxin levels by inhibiting biosynthesis of IAA (15), and this also needs to be investigated further. Another possibility is that ethylene treatment alters extractability of auxins from the tissues.

The significance of the influence of ethylene on IAA levels, and IAA on ethylene levels could be related to a feedback mechanism regulating growth, as suggested by Burg and Burg (3) and Lieberman and Kunishi (9).

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LITERATURE CITED

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