Plant Physiol. (1967) 42, 1803-1806

Short Communication

Effect of Ethylene and Gibberellic Acid on Auxin Synthesis in Plant Tissues[1, 2]

Jack G. Valdovinos, Leland C. Ernest[3], and Egbert W. Henry
Department of Biology, Wayne State University, Detroit, Michigan 48202

Received August 23, 1967.

Treatment of plant tissues with gibberellic acid leads to increased levels of auxin and stem elongation in several plant species (7, 13). Pretreatment of plant tissues with ethylene is known to decrease levels of diffusible auxin (5, 10, 20) as well as cell elongation (3, 4). Recent evidence has indicated that a significant influence of gibberellin on auxin levels and growth processes is through its effect on auxin synthesis in plant tissues (8, 17). The possibility that ethylene may also regulate auxin levels in plant tissues by affecting the synthesis of auxin is suggested by the literature (4, 5, 10, 11, 20). Further support of this proposal is suggested by papers (1, 10, 20) which show ethylene to have no direct effect on the polar transport of auxin. Another indication that ethylene, in addition to gibberellin, may be involved in the regulation of auxin synthesis, lies in the fact that gibberellin and ethylene have opposing effects on the elongation of hypocotyls of lettuce seedlings (14). The possibility that ethylene affects the formation of auxin from tryptophan was investigated and the results of the experiments are presented in this paper.

Pea seedlings (*Pisum sativum* L. varieties Alaska and Little Marvel) were grown under 14 hours per day of light at a temperature of 23 ± 2° during the day and 20° at night. The seedlings were grown in a medium of Perlite and vermiculite (1:1) saturated with half-strength Hoagland's nutrient medium. Eighteen hours before harvesting the tissue for tryptophan and auxin studies, the plants were placed in plastic or glass chambers and ethylene (25 μl/l) was added by means of a hypodermic syringe. Seedlings receiving gibberellic acid were treated in the apical region with 0.1 ml of 10 μM gibberellic acid in 0.05 % Tween-20.

Assays for tryptophan-^{14}C conversion and IAA-^{14}C destruction by cell free preparations of the apical tissue were conducted as described in an earlier report (17), except that Penicillin G at a final concentration of 0.25 mM served as the antibiotic during a 3 hour incubation period. L-Tryptophan-1-^{14}C (0.1 μc) was supplied with the enzyme preparation at a final concentration of 0.2 mM and a specific activity of 0.125 mc/mmole. Indoleacetic acid-1-^{14}C (0.07 μc) was included in other incubation flasks at a final concentration of 1.5 μM and a specific activity of 12 mc/mmole. This is a level of auxin within the concentration range of diffusible auxin present in *Coleus* stem tissue as reported earlier (12). The conversion of tryptophan to auxin by cell free preparations of *Coleus* tissue was studied by incubating 1 ml of heated (90°) or unheated enzyme preparation with 1 ml of tryptophan (1.54 mg/ml) and 0.1 ml of 10 mM Penicillin G. The tryptophan had been purified previously with peroxide-free ether by refluxing for 24 hours in a Soxhlet extractor. A portion of the auxin formed during the 2 hours of incubation in the dark, was trapped in 13 agarose blocks (1 %), each of a volume of 8 μl, which had been included in each incubation flask. The auxin content of the blocks was then determined using the *Avena* curvature bioassay. Diffusible auxin from the apical bud regions of the stems was collected in agarose blocks over a 2 hour period during which the tissue was exposed to diffuse light. Where comparisons are made between diffusible auxin levels and rates of tryptophan-1-^{14}C conversion with the release of $^{14}CO_2$, the enzyme preparations were made from the same apical bud regions at the end of the 2 hour diffusion period. In each experimental condition the diffusible auxin was collected from the apical bud regions of 10 *Coleus* plants, 12 Alaska pea seedlings, and 16 dwarf pea seedlings. Standard errors are included in table I and the t-test (15) was employed in evaluating significant differences between treatments. Studies of tryptophan-1-^{14}C conversion with the release of $^{14}CO_2$ were repeated a minimum of 5 times with each type of plant tissue. Auxin determinations were repeated 3 times.

The possibility that the tryptophan conversion observed in these experiments is due to bacterial

[1] Supported in part by Grants No. GB-3870 and No. GB-6722 from the National Science Foundation.

[2] Publication No. 193, Department of Biology, Wayne State University.

[3] Present address: Department of Biology, Eastern Nazarene College, Quincy, Massachusetts.

contamination is discounted for the following reasons: 1) ultrastructural studies which reveal no invasion by bacteria of tissues of intact plants (6); 2) the washing and surface sterilization procedures followed in this laboratory; 3) the fact that the rupture of bacterial cells by the grinding methods used is unlikely; 4) the magnitude and duration of forces utilized in preparing a cell free extract; 5) the conduction of assays in sterilized flasks in the presence of antibiotics; 6) the fact that rates of conversion are too consistent and too high to be attributed to chance contamination; 7) the fact that the hormonal influences observed are more likely to be characteristic of metabolism in higher plant cells than in bacteria.

Table I (expt 1) illustrates the effect of ethylene pretreatment on tryptophan conversion and auxin levels in *Coleus*. Cell free preparations from treated tissue are less active in decarboxylating tryptophan (or an indole intermediate with a 3-carbon side chain) than enzyme preparations from untreated tissue. Corresponding to the reduction in tryptophan-1-^{14}C conversion with the release of $^{14}CO_2$, there is a decrease in conversion of tryptophan to auxin by cell free preparations of the same tissue sample. In other experiments where tryptophan-3-^{14}C was incubated with cell free preparations from untreated *Coleus* tissue, an acidic indole substance was extracted with methylene chloride and chromatographed. Elution from the thin layer plate at the band corresponding to authentic IAA yielded a level of radioactivity of approximately 20% the radioactivity released in the form of $^{14}CO_2$ from tryptophan-1-^{14}C. Further experiments are in progress to determine the effect of ethylene on the yield of this fraction. Destruction of IAA-1-^{14}C with the release of $^{14}CO_2$ by cell free preparations also of the same tissue sample, is decreased rather than enhanced. In other experiments no effect of ethylene on IAA decarboxylation by *Coleus* tissue was observed. Ethylene was reported earlier to have no effect on the destruction of auxin in stem sections of Alaska pea seedlings (4) and in cotton plants (11). In an experiment parallel to that described above, both tryptophan-1-^{14}C conversion with the release of $^{14}CO_2$ and diffusible auxin levels are observed to be decreased by ethylene treatment. In this experiment, a larger portion of the apical region of the *Coleus* plant was used in order to detect the auxin diffusing from the stem (the tissue included the first pair of small unfolded leaves in addition to the apical bud region). The apparent higher conversion rate of tryptophan in this experiment is thus due to a higher ratio of tissue weight to buffer volume.

The effect of ethylene pretreatment on tryptophan-1-^{14}C conversion with the release of $^{14}CO_2$ and on levels of diffusible auxin in normal and dwarf pea seedlings is illustrated in table I (expts 2 and 3). Corresponding to the decrease in conversion rate of the auxin precursor, lower levels of diffusible auxin are observed in these tissues. In the dwarf pea, the rate of tryptophan conversion in the untreated tissue is 5.3 times that in ethylene treated tissue while the level of diffusible auxin is 5.5 times that in the treated tissue. When gibberellic acid is applied to the dwarf pea seedling, increased levels of tryptophan conversion and diffusible auxin are observed. The tryptophan conversion rate in the treated tissue is 1.7 times that in the control while the level of diffusible auxin is 1.6 times that in the untreated tissue. A combination ethylene and gibberellic acid pretreatment restores both the conversion rate of the auxin precursor and the amount of diffusible auxin to levels comparable to those observed in the untreated tissue.

As is seen in these experiments, pretreatment of *Coleus* plants with ethylene appears to reduce the level of auxin diffusing from the apical region by a greater percentage than the percentage by which the conversion of tryptophan to auxin is decreased. This discrepancy between percentages of reduction of auxin synthesis and transport may indicate that a linear proportionality between the 2 phenomena does not exist in *Coleus* tissue; thus smaller changes—percentage-wise—in auxin synthesis could result in large changes in auxin transport. Another point which cannot be determined from the observations is whether ethylene treatment results in a more complete decrease in auxin formation than is reflected in these experiments. Since ethylene had no effect on the release of $^{14}CO_2$ from tryptophan-1-^{14}C when it was added directly to the incubation flasks containing enzyme preparations from *Coleus* tissue, it would appear that ethylene may affect the formation of the auxin precursor converting enzyme system. Thus some of the auxin synthesized may have been formed by enzymes existing prior to the action of ethylene.

The observations reported herewith strongly indicate that ethylene decreases levels of diffusible auxin in plant tissues through its influence on auxin synthesis. Leopold and Lam (9) demonstrated earlier the necessity of a continuous supply of auxin in order to maintain an efficient auxin transport system in the stem of the sunflower seedling. The possibility that the production of auxin may be reduced in the presence of ethylene was considered earlier (3) not to be a requisite for ethylene action in cell elongation in pea stem tissue, since in the pea stem assay the site of auxin production has supposedly been removed. However, more recent investigations indicate that auxin is synthesized in such tissues as petioles of *Coleus* (18), in excised stem segments of both normal and dwarf pea seedlings (16), and in the stem tissue of decapitated and intact sunflower seedlings (2, 19). In view of these more recent data, it would appear that plant tissues other than leaves and stem and root apical regions have at least some capacity to syn-

Table I. *Effect of Ethylene and Gibberellic Acid on Tryptophan Conversion by Cell Free Preparations and Diffusible Auxin in Stem Apical Bud Tissues of* Coleus, *Alaska Pea and Little Marvel Dwarf Pea Seedlings*

In expt 1, 0.2, 1, and 2 μM IAA gave 6.8, 27.5, and 34.5° of curvature, respectively. In expt 2, 0.1, 0.3, 0.6, 1, and 2 μM IAA gave 1.8, 5.3, 13.0, 25.5, and 31.2°, respectively. In expt 3, 0.2, 0.6, 1, and 2 μM IAA gave 3.3, 15.2, 26.2, and 34.3°, respectively

Pretreatment	TTP-1-^{14}C or IAA-1-^{14}C Conversion dpm obs	dpm/g fr wt	Auxin levels Deg curv	$\times 10^{-2}$ nmoles/g fr wt (IAA equiv.)
Expt 1 *Coleus* plants[†]				
	TTP conversion		auxin formation	
Control	6470	26,100	14.8 ± 1.0	858
Ethylene	4335	17,500	11.4 ± 0.6*	594
	IAA destruction			
Control	1360	11 000	xxx	xxx
Ethylene	1145	9,245	xxx	xxx
	TTP conversion		diffusible auxin	
Control	9970	23,200	9.5 ± 0.8	1.7
Ethylene	6490	15,100	1.7 ± 0.5**	0.5
Expt 2 Alaska peas, 4 wks old				
	TTP conversion		diffusible auxin	
Control	1405	2640	22.6 ± 1.1	11
Ethylene	488	915	8.7 ± 1.5**	5
Expt 3 Little Marvel dwarf peas, 3 wks old				
	TTP conversion		diffusible auxin	
Control	340	850	16.1 ± 1.5	11
Ethylene	65	162	1.4 ± 0.4**	2
GA_3	591	1478	22.0 ± 1.2**	18
Ethylene and GA_3	352	880	16.1 ± 1.3	13

* Significantly different from control at the 98 % level.
** Significantly different from control at the 99 % + level.
† Grown under greenhouse conditions.

thesize auxin. It is reasonable to assume, therefore, that ethylene would influence the endogenous auxin formation system in such intact or excised tissues in a manner similar to its effect on the apical bud of the stem as reported here. This action of ethylene may contribute to the onset of senescence in certain plant tissues.

During the time this report was in press, 2 papers have appeared which relate directly to this investigation. Burg and Burg [Plant Physiol. (1967) 42: 1224–28] present further evidence that ethylene does not directly affect auxin levels in plant tissues by influencing polar transport or auxin destruction. Reed and Crecelius [Plant Physiol. (1967) 42: 1303–06] report that they were unable to recover known intermediates of auxin formation from labeled tryptophan incubated with enzyme preparations from pea seedling stem tissue. They suggest that tryptophan decarboxylation may yield oxindole derivatives of IAA. We feel that the yield of such derivatives from tryptophan would be less than the yield of auxin, in view of this report and our earlier experiments (17) which included a comparison of the release of $^{14}CO_2$ from carbon atoms 1 and 2 of the side chain of tryptophan. We also do not feel that indolepyruvic acid can be ruled out as a possible intermediate because of the failure to extract the substance from incubations containing tryptophan-1-^{14}C. Until the enzymes involved in tryptophan conversion can be purified, tryptamine also cannot be eliminated as an intermediate in auxin formation. Tryptamine has been observed in this laboratory to inhibit markedly the release of $^{14}CO_2$ from tryptophan-1-^{14}C when added to flasks containing *Coleus* enzyme preparations. Therefore, any experimental procedure, e.g. N_2 atmosphere, which would prevent the oxidation of the amine intermediate could make tryptophan decarboxylation appear O_2 dependent.

Literature Cited

1. Abeles, F. B. 1966. Effect of ethylene on auxin transport. Plant Physiol. 41: 946–48.
2. Bednarz, R. M., L. C. Ernest, and J. G. Valdovinos. 1967. Enhancement of auxin precursor conversion by gibberellic acid and stem elongation in the sunflower seedling. Plant Physiol. 42: S-30.
3. Burg, S. P. and E. A. Burg. 1965. Ethylene action and the ripening of fruits. Science 148: 1190–96.
4. Burg, S. P. and E. A. Burg. 1966. The interaction between auxin and ethylene and its role in plant growth. Proc. Natl. Acad. Sci. U. S. 55: 262–69.
5. Guttenberg, H. von and E. Steinmetz. 1947. The effects of ethylene on growth hormone and growth. Pharmazie 2:17–21.

6. JENSEN, T. E. AND J. G. VALDOVINOS...Fine structure of abscission zones. Planta: 77: 298–318.
7. KURAISHI, S. AND R. M. MUIR. 1962. Increase in diffusible auxin after treatment with gibberellin. Science 137: 760–61.
8. KURAISHI, S. AND R. M. MUIR. 1964. The mechanism of gibberellin action in the dwarf pea. Plant Cell Physiol. 5: 259–71.
9. LEOPOLD, A. C. AND S. L. LAM. 1962. The auxin transport gradient. Physiol. Plantarum 15: 631–38.
10. MICHENER, H. D. 1938. The action of ethylene on plant growth. Am. J. Botany 25: 711–20.
11. MORGAN, P. W. AND H. W. GAUSMAN. 1966. Effects of ethylene on auxin transport. Plant Physiol. 41: 45–52.
12. MUIR, R. M. AND J. G. VALDOVINOS. 1965. Gibberellin and auxin relationships in abscission. Plant Physiol. 40: xxvi.
13. NITSCH, J. P. 1957. Growth responses of woody plants to photoperiodic stimuli. Proc. Am. Soc. Hort. Sci. 70: 512–25.
14. SCOTT, P. C. AND A. C. LEOPOLD. 1967. Opposing effects of gibberellin and ethylene. Plant Physiol. 42: 1021–22.
15. SNEDECOR, G. W. 1956. Statistical Methods. 5th edition. Iowa State University Pres, Ames, Iowa.
16. TANIMOTO, E., N. YANAGISHIMA, AND Y. MASUDA. 1967. Effect of gibberellic acid on dwarf and normal pea plants. Physiol. Plantarum 20: 291–98.
17. VALDOVINOS, J. G. AND L. C. ERNEST. 1966. Gibberellin-enhanced CO_2 release from tryptophan-1-^{14}C in plant apical tissue. Plant Physiol. 41: 1551–52.
18. VALDOVINOS, J. G. AND J. E. PERLEY. 1966. Metabolism of tryptophan in petioles of *Coleus*. Plant Physiol. 41: 1632–36.
19. VALDOVINOS, J. G. AND L. C. ERNEST. 1967. Effect of gibberellic acid and Cycocel on tryptophan metabolism and auxin destruction in the sunflower seedling. Physiol. Plantarum 20: 682–87.
20. VAN DER LAAN, P. A. 1934. Der Einfluss von Aethylen auf die Wuchsstoffbildung bei *Avena* und *Vicia*. Rec. Trav. Botan. Neerl. 31: 691–742.