INTERACTION OF PLANT GROWTH REGULATORS IN AUXIN-INDUCED ETHYLENE PRODUCTION

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Ethylene is produced by a number of plant tissues and affects many physiological as well as biochemical processes in plant tissues (1-3). It has been suggested that endogenous auxin level regulates endogenous ethylene production (4) and exogenously applied auxin stimulates ethylene production by inducing the enzymes involved in the formation of the gas (5-8).

While ethylene production by plant tissue is greatly stimulated by auxin, it has also shown that other plant growth regulators affect ethylene production. Bean stem segments excised from plants which had been sprayed with gibberellin produced ethylene twice the amount produced from unsprayed control plants (4). Kinetin at concentration of 10^{-8} to 10^{-4} M stimulated ethylene production in etiolated pea seedlings (9).

Interaction of auxin, kinetin and gibberellin in ethylene production has been reported by Fuchs and Lieberman (9) with etiolated pea seedlings and stem segments. They have shown that in stem segments excised from 6-day-old seedlings, ethylene production was considerably stimulated if kinetin was added with axuin. They have also shown that gibberellin had no effect even in the presence of auxin or kinetin.

Those results clearly indicate that ethylene production by plant tissue is regulated by plant growth substances and there is an apparent interaction among them. Although many workers have interested in these phenomena, their attentions have mainly directed to physiological effects of ethylene on plant growth, and only a few biochemical studies on ethylene synthesis modified by plant growth regulators have been reported.

It is the purpose of the present paper to elucidate the effects of plant growth regulators on auxin-induced ethylene production.

MATERIALS AND METHODS

Plant material

Etiolated seedlings of mungbean (*Phaseolus aureus* Roxb.) were grown, as previously described (7), on a 0.4 % agar-gel bed at 25°C in the dark. After 72 hr, when hypocotyl length reached 30 to 35 mm, a 6 mm-long hypocotyl segment below the cotyledonary hook was excised from each seedling.

Incubation

Ten segments (0.14 g) were placed in a 25 ml-glass vial containing

0.5 ml of an incubation medium. Fifty mM phosphate buffer, pH 6.8, containing 50 μg/ml chloramphenicol was used as incubation buffer. Potassium indoleacetate was used as auxin. Plant growth regulators were dissolved in the above buffer solution. The glass vial was closed with a silicon rubber stopper and incubated at 30°C in the dark. After 12 hr incubation, the ethylene content in the gas phase was measured with a gas chromatograph.

Assay of relative levels of free auxin in the tissue

Twenty hypocotyl segments were incubated at 30°C for 9 hr in a Petri dish (3 cm in diameter) with 1 ml of 0.1 mM Carboxyl-^{14}C-IAA* (0.1 μCi) in the incubation buffer with or without other plant growth regulators. At the end of the incubation period, segments were thoroughly washed with ice-chilled water and homogenized with 80 % ethanol in a glass homogenizer. The homogenate was centrifuged at 3,000×g for 10 min, and precipitate was extracted with 80 % ethanol two more times. The combined ethanol extract was concentrated *in vacuo* to dryness, and the residue was dissolved in 1 ml of 80 % ethanol. The ethanol solution was then chromatographed on Toyo filter paper No. 51 with isopropanol-ammonium hydroxide-water (8 : 1 : 1, v/v). Zones corresponding to free IAA (Rf 0.61) and IAAsp** (Rf 0.31) were cut out under UV light and radioactivities in these sections were measured with a liquid scintillation spectrometer.

RESULTS

Effect of auxin concentration on ethylene production

Without auxin, mungbean hypocotyl segments produced small amounts of ethylene. One μM auxin supplied in the incubation buffer increased ethylene production and as auxin concentration was raised, ethylene production progressively increased (Fig. 1). However, the responce of segments to auxin was maximal at 0.5 mM auxin.

Effects of plant growth regulators on auxin-induced ethylene production

Fig. 2 indicates that kinetin alone stimulated ethylene production though its effect was not striking as the auxin effect. The effect of kinetin was also dependent upon kinetin concentrations. In the absence of added auxin, 0.1 mM kinetin increased ethylene production 10-fold during 12 hr. When 0.1 mM or lower of auxin was present, kinetin further stimulated auxin-induced ethylene production, and a significant synergism of kinetin and auxin was observed. However, the magnitude of stimulation by kinetin to kinetin-free control decreased as auxin concentration was increased, and in the presence of 0.5 mM auxin, kinetin did not show any stimulative effect.

* IAA: Indoleacetic acid.
**IAAsp: Indoleacetylaspartate.

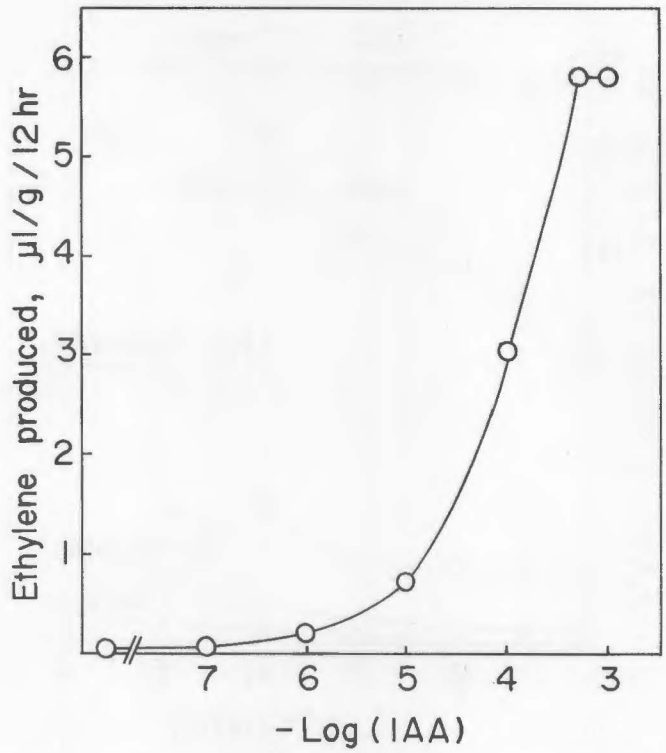

Fig. 1. Ethylene production by hypocotyl segments induced with various concentration of auxin (IAA).

Ethylene production induced by auxin was also stimulated by gibberellin (Fig. 3), although gibberellin effect was smaller than kinetin effect. Presence of 0.1 μM gibberellin stimulated about 30 % over control value, and no further stimulation was observed even higher concentrations of gibberellin were used. As in the case of kinetin, 0.5 mM auxin induced ethylene production was not affected by gibberellin at any concentrations used. Gibberellin, however, did not clearly stimulate endogenous ethylene production (Fig. 3).

Fig. 4 indicates that abscisic acid at concentration over 1 μM inhibited auxin-induced ethylene production. Rate of inhibition of ethylene production at given abscisic acid concentration was independent upon auxin concentration, thus, 0.01, 0.1 and 1 mM abscisic acid achieved about 15, 55 and 77 % inhibition, respectively, in the presence either 0.01, 0.1 and 0.5 mM auxin. Abscisic acid had little effect on endogenous ethylene production (Fig. 4).

Effects of plant growth regulators on endogenous levels of free IAA in the tissue

It has been suggested that the rate of ethylene production by pea

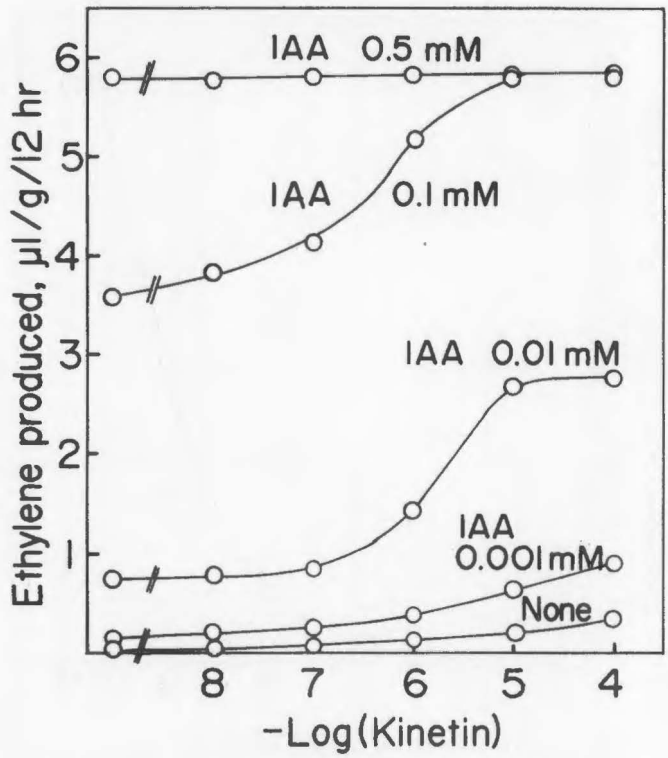

Fig. 2. Stimulation by kinetin of auxin-induced ethylene production by mungbean hypocotyl segments.

stems after application of auxin closely followed the levels of free auxin in the tissue (10). Since, in the present investigation too, it was considered that auxin-induced ethylene production was regulated by endogenous levels of IAA in the tissue, the relative levels of free IAA in the tissue after application of plant growth regulators were investigated. Although, kinetin and gibberellin greatly stimulated ethylene production, the relative level of free IAA in the tissues treated by kinetin or gibberellin was only slightly higher than that of control tissue (Table 1).

TABLE 1.

Effects of plant growth regulators on the level of free IAA and IAAsp formation in mungbean hypocotyl segments.

Additions	Free IAA cpm	IAAsp cpm
None	3,410	15,822
Kinetin (0.1 mM)	4,356	15,496
Gibberellin (0.1 mM)	4,298	16,226
Abscisic acid (0.1 mM)	6,018	18,412

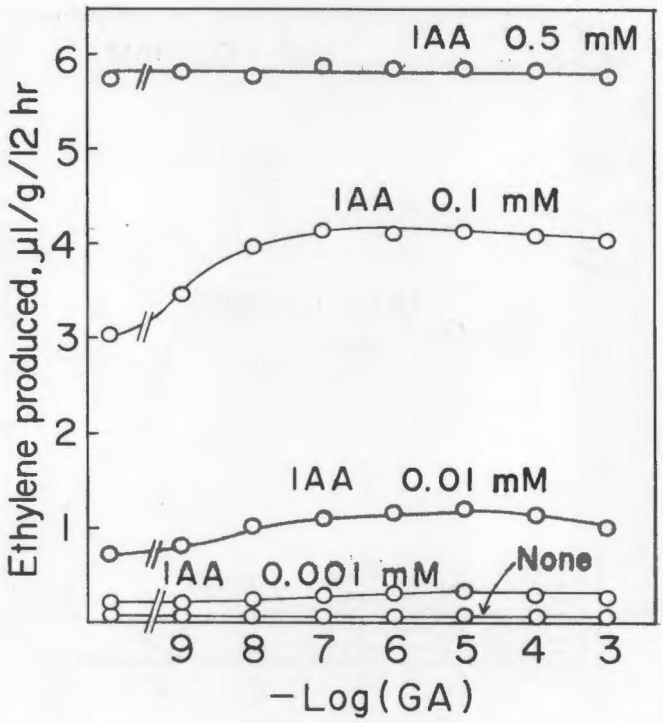

Fig. 3. Stimulation by gibberellin (GA) of auxin-induced ethylene production by mungbean hypocotyl segments.

Treatment with abscisic acid reduced ethylene production (Fig. 4) but the treatment rather increased the relative level of endogenous IAA in the tissue and the formation of IAAsp.

DISCUSSION

The magnitude of the inductive increase in ethylene production is dependent on axuin concentration (Fig. 1). However, the effect of auxin was maximal at 0.5 mM.

Effects of plant growth regulators on auxin-induced ethylene production as well as on relative free auxin level in the tissue revealed some interrelationships among actions of auxin and other plant growth regulators. The data with kinetin indicate that the effect of kinetin is dependent upon auxin and maximal level of ethylene production appears to be regulated by auxin level. This is clearly shown in the presence of 0.5 mM auxin where kinetin does not show any stimulative effect (Fig. 2). Capacity of ethylene production induced by 0.5 mM auxin seems to be maximal for the hypocotyl segments and this is consistent with the fact that auxin level higher than 0.5 mM no further stimulation is observed (Fig. 1). Stimulation of endogenous ethylene production by

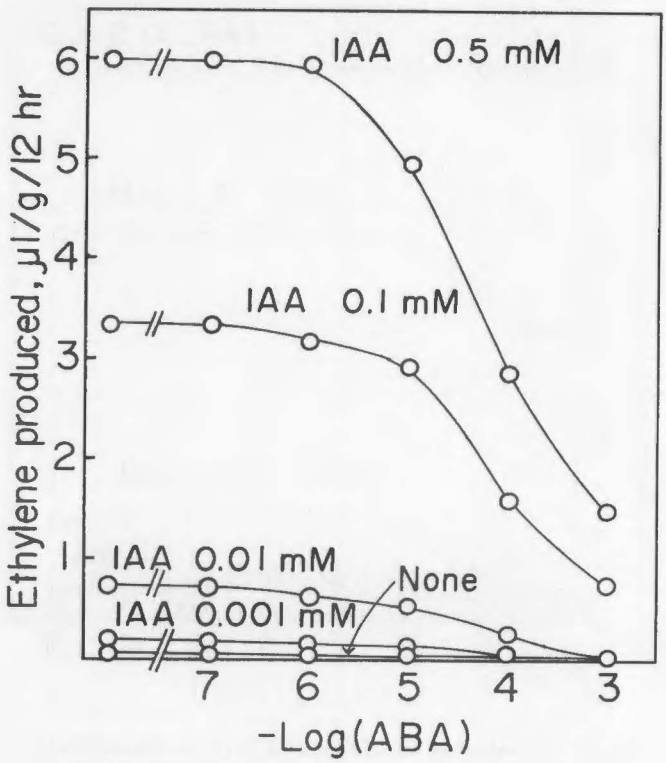

Fig. 4. Effect of abscisic acid (ABA) on auxin-induced ethylene production by mungbean hypocotyl segments.

kinetin alone may be considered to be synergistic effect of kinetin with endogenous auxin.

Although kinetin treatment slightly increased relative endogenous auxin level in the tissue, the great stimulative effect of kinetin on ethylene production was not explained by the increase in relative endogenous auxin level. Therefore, it is considered that the stimulative effect of kinetin may be to magnify the auxin action.

Abeles (5) showed that auxin-induced ethylene production by etiolated bean hypocotyl sections was inhibited by actinomycin D and to a lesser extent by puromycin. Ethylene production by pea seedlings induced by auxin is also inhibited by cycloheximide and actinomycin D (6). We also showed that auxin-induced ethylene production by mungbean hypocotyl segments was inhibited by cycloheximide, puromycin and actinomycin D (7,8). Those results suggest the involvement of RNA and protein synthesis in the induction of ethylene evolution by auxin. Since kinetin stimulates RNA synthesis (11,12) and kinetin effect seems to magnify the auxin action on induction processes, it can be assumed that

the effect of kinetin on ethylene production is resulted from the stimulation of RNA synthesis caused by auxin.

In contrast to the experimental results with pea seedlings (9), gibberellin stimulated auxin-induced ethylene production by mungbean hypocotyl segments. Apparent effect of gibberellin is similar to that of kinetin although degree of stimulation is not as great as kinetin and gibberellin effect was saturated at a lower concentration (Fig. 3). It has been suggested that gibberellin increases diffusible auxin in the tissue (13). As shown in Table 1, gibberellin increased the relative free auxin level about 26 % in the presence of 0.1 mM auxin. This may account for 30 % increase of ethylene production by gibberellin in the presence of 0.1 mM auxin (Fig. 3). If, however, gibberellin generally increases free auxin level in tissue, endogenous ethylene production in mungbean hypocotyl should also be increased by gibberellin, which was not clearly observed. The author assumes that a slight stimulation of endogenous ethylene production by gibberellin was not detected due to the sensitivity of the gas chromatograph.

Auxin-induced ethylene production is inhibited by abscisic acid, and the inhibition is not competitive with auxin. This means that abscisic acid acts probably independent of auxin action. This assumption is further supported by the fact that abscisic acid inhibited ethylene production though it significantly increased the relative level of endogenous free IAA in the tissue (Table 1). A number of experiment indicated that abscisic acid inhibited RNA synthesis (14–16). Therefore, it is assumed that abscisic acid inhibited the synthesizing process of ethylene producing system, presumably the synthesis of RNA.

SUMMARY

Auxin induced an increase in the rate of ethylene production by hypocotyl segments of etiolated mungbean seedlings. Kinetin stimulated auxin-induced ethylene production as well as endogenous ethylene production. Ethylene production induced by auxin was also stimulated by gibberellin, although gibberellin effect was smaller than kinetin effect. Abscisic acid inhibited auxin-induced ethylene production, and the inhibition is not competitive.

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