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Specificity of Auxin Action on Ethylene Production in Corn Coleoptile Segments

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옥수수 (*Zea mays* L.) 자엽초 절편에서 에틸렌 생성에 대한 오옥신의 작용 특성

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ABSTRACT

The ability of several auxin analogs to induce ethylene production was tested in the corn coleoptile. The synthetic auxins 1-naphthaleneacetic acid (1-NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) had strong stimulatory effects on ethylene induction surpassing that of IAA. Both 2-naphthalaneacetic acid (2-NAA) and 2,6-dichlorophenoxy acetic acid (2,6-D), structural analogs of these auxins, respectively, were found to be inactive. Treatment with NPA, a strong inhibitor of polar auxin transport, led to drastic increase in IAA-induced ethylene production while it has no effect on ethylene production induced by 1-NAA. A positive correlation existed between intracelluar auxin level and ethylene production.

INTRODUCTION

Several synthetic auxins cause many of the physiological responses specific to IAA and are also transported polarly in plant tissues. Their structure-activity relationships (Katekar *et al.*, 1986) provide valuable information for the studies on the nature of auxin action, because auxin binding to the specific receptor is thought to be prerequisite to auxin action. Polar transport of auxin also involves specific protein carriers in the plasmalemma (Hertel, 1985). However, problems of the primary site of auxin action are controversal (Vesper and Kuss, 1990; Venis *et al.*, 1990), and moreover, multiple auxin receptor sites have been reported (Ray *et al.*, 1977; Firn, 1986).

By using NPA, the specific inhibitor of polar auxin transport (Thomson and Leopold, 1974), whose action site is considered to be the putative auxin efflux carrier in the plasmalemma (Depta *et al.*, 1983), we investigated actions of various auxins on the induction of ethylene production.

MATERIALS AND METHODS

Plant material. Pre-soaked corn (*Zea mays* L.) seeds were planted on wet paper towels in plastic trays and placed vertically in the dark at 28℃ with saturated humidity. When seedlings were 4-5 day old, subapical coleoptile segments of 1 cm in length were cut with a double blade cutter and primary leaves were removed.

Radiochemicals. (5-^{3}H)-IAA (28 Ci/mmole) and (^{14}C)-NAA (50.6 mCi/mmole) were purchased from CEA (Gif-sur-Yvette, France) and Amersham, respectively.

Ethylene production. Ethylene production was measured according to Kang *et al.* (1971). Ten subapical coleoptile segments (1 cm) were incubated with 3 m*l* buffered solution (10 mM sodium phosphate, pH 6.8, 1% sucrose) in a 25 m*l* Erlenmyer flask sealed with silicon rubber cap. After 18 h incubation in the dark with gentle shaking, 1 m*l* air samples were taken from the flask with a hypodermic syringe and ethylene production was mea-

Table 1. Ethylene production induced by various auxin analogs in corn coleoptile segments

Auxin analogs (3 μM)	Ethylene production* (nl/g.f.w)
Control	32
IAA	163
2,4-D	325
1-NAA	215
2-NAA	42
2,6-D	46
PCIB	53
PAA	38

*Ten 1 cm coleoptile segments were incubated with 3 μM each and ethylene production was measured after 18 h incubation.

sured with gas chromatography (Simadzu, GC-3BF, flame inonization detector, alumina column).

Net uptake of ^{3}H-IAA. Net uptake of auxin was measured as described previously by Edwards and Goldsmith (1980). Subapical corn coleoptile tissues were cut into 1 mm slices with a multibladed cutter. Twenty slices were incubated in 1 m*l* sodium phosphate buffer (10 mM, pH 5) containing 8.9×10^{-9} M ^{3}H-IAA or 9.8×10^{-7} M ^{14}C-NAA in the presence of NPA at various concentrations in a 20 m*l* vial. At the end of an uptake period (ca. 30 min) the medium was rapidlly removed and slices washed twice with cold buffer under reduced pressure. Ten slices were transferred to a scintillation vial and the radioactivity counted.

RESULTS AND DISCUSSION

Physiological concentrations of IAA and other auxins stimulate ethylene production in a number of tissues (Lieberman, 1979). Among various auxins tested, 2,4-D and 1-NAA were shown to have a strong activity of stimulating ethylene formation surpassing the natural auxin IAA in corn coeoptile segments (Table 1). Lack of activity of 2-NAA and 2,6-D, structural analogs of 1-NAA and 2,4-D, respectively, indicate that those auxin analogs inactive in growth are likewise ineffective to induce ethylene production, suggesting a primary action common to both physiological responses.

Typical dose-response relationships in which ethylene production is proportionally enhanced as the auxin concentration increase up to 10^{-4} M were observed for both IAA and 1-NAA (Fig. 1A and 1B). Further increase in

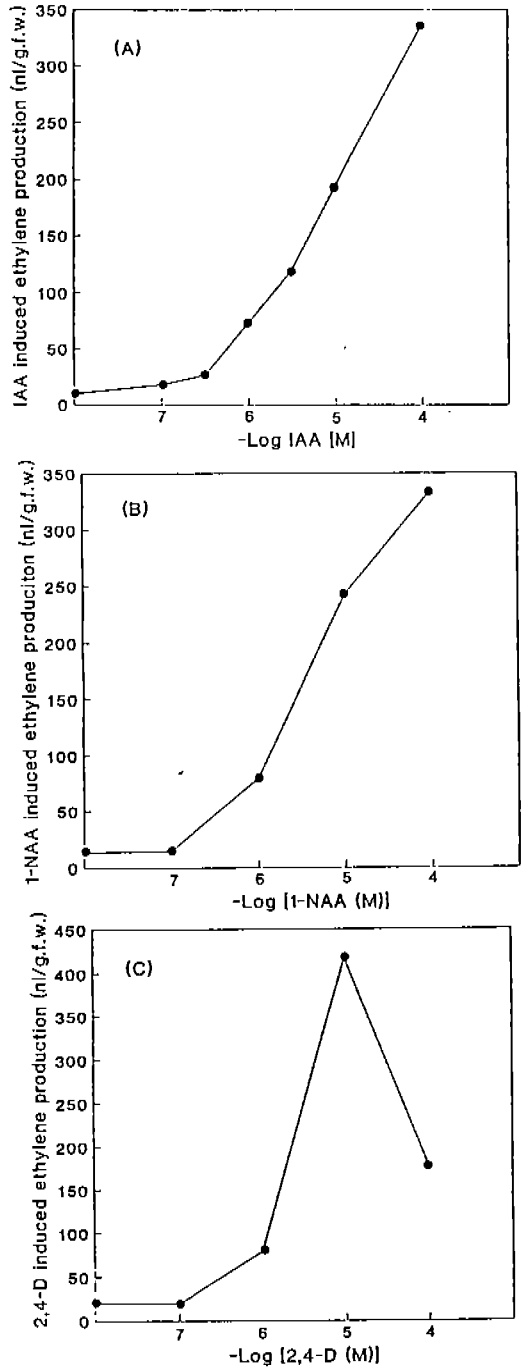

Fig. 1. Dose-response curve for ethylene production in corn coleoptile segments. Ten 1 cm subapical segments were incubated with IAA (A), 1-NAA (B) or 2,4-D (C), respectively at various concentrations. Ethylene production was measured after 18 h incubation. Data represent average values of three duplicate experiments.

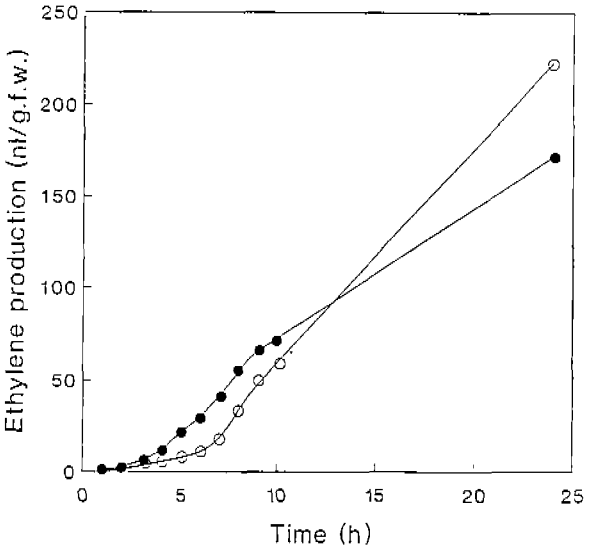

Fig. 2. Time course of ehylene production induced by IAA (●) or 1-NAA (○) at 10 μM concentration. Ethylene production was measured every 1 h. Data represent average values of two duplicate experiments.

the auxin concentration was found to be supraoptimal (data not shown) in corn coleptile segments as indicated by Moore (1989). The sharp optimum at 10^{-5} M 2,4-D as shown in Fig. 1C is probably due to a possible toxic effect of this auxin at concentrations above 10^{-5} M.

Although both synthetic auxins and IAA have simillar does-response relationships in stimulating ethylene production, time course data indicate that they show different kinetics of ethylene production (Fig. 2). Compared with IAA-induced ethylene production, 1-NAA application brought about initial delay followed by a steady increase in ethylene production until 24 h. The stability of ethylene producing system induced by IAA in the coleoptile as shown in Fig. 2 is quite a contrast to the pea internode system where ethylene production rapidly declines after 12 h, and rather resembles that induced by 2,4-D in the pea tissue (Kang *et al.*, 1971). According to Kang *et al.* (1971), ethylene production requires the continual presene of auxin at a physiologically active concentration, and thus differences in the stability of ethylene producing systems may reflect differences in cellular metabolism of these auxins.

NPA, a strong inhibitor of auxin transport, has been known to stimulate net uptake of auxin by specifically inhibiting auxin efflux out of the cell (Sussman and Goldsmith, 1980). NPA applied to tissue segments leads to

Table 2. Effect of NPA on IAA-induced ethylene production and cell elongation in corn coleoptile segments

IAA conc.	Ethylene production (nl/g.)*		Increase in length (mm)*	
	(−) NPA	(+) NPA**	(−) NPA	(+) NPA
0.3 μM	21	65	2.2	3.6
10 μM	196	330	3.2	2.6

*Ten 1 cm coleoptile segments were incubated with IAA at concentrations indicated in the presence or absence of 10 μM NPA. Ethylene production and increase in length were measured after 18 h incubation. Data repesent average values of three duplicate experiments.
**10 μM NPA.

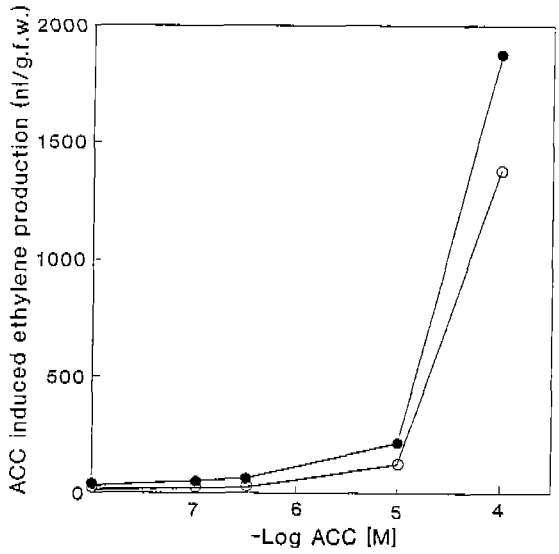

Fig. 3. Effect of NPA on ACC induced ethylene production in corn coleoptile segments. Ethylene production was measured after 18 h incubation in the presence (○) or absence (●) of 10 μM NPA. Data represent average values of two duplicate experiments.

growth promotion as a result of elevated intracellular auxin level (Vesper *et al.*, 1987). In our present work, a remarkable enhancement of ethylene production was brought about by NPA (Table 2). NPA alone, however, had negligible effect on the basal level of ethylene production (data not shown), indicating that the NPA effect was auxin dependent. This was supported by the fact that NPA effect on ACC-based ethylene production was insignificant (Fig. 3).

It is noticed that growth was rather inhibited by NPA at a high IAA concentration (10 μM) while NPA stimula-

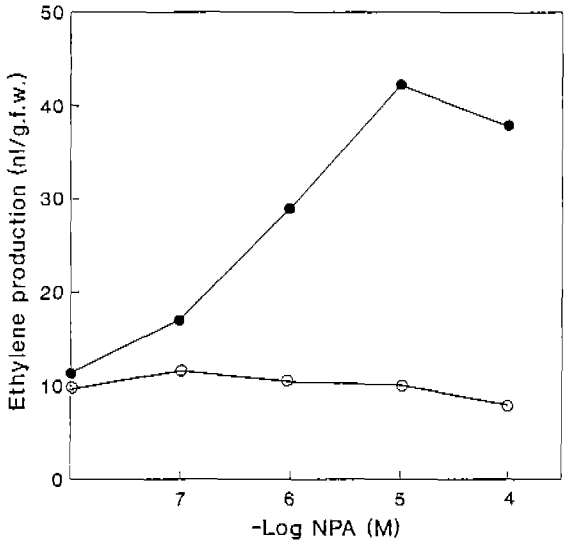

Fig. 4. Effect of NPA on auxin-induced ethylene production in corn coleoptile segments. Ten, 1 cm subapical segments were incubated with 1 μM IAA (●) or 1-NAA (○) in the presence of NPA. Ethylene production was plotted against NPA concentrations after 18 h incubation. Data represent average values of three duplicate experiments.

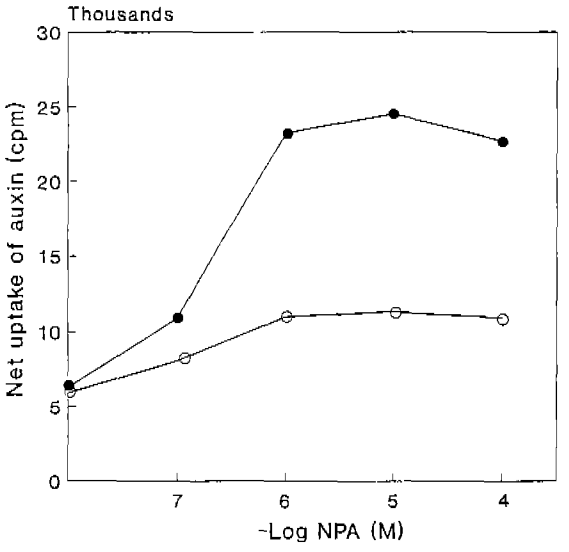

Fig. 5. Effect of NPA on net uptake of auxin by corn coleoptile slices (1 mm). Net uptake of ^{3}H-IAA (●) or ^{14}C-NAA (1-NAA, ○) was measured in the presence of NPA at pH 5. Radioactivity in the tissue slices was plotted against NPA concentrations after 30 min incubation. Data represents average values of two duplicate experiments.

ted ethylene production at the same concentration of IAA. Stimulating effect of NPA on both growth and ethylene production was observed at a low IAA concentration (0.3 μM). Does-response curve for growth with an optimum value at a lower concentration of auxin (Vesper and Evans, 1979) than that for ethylene production (Fig. 1) may explain the difference in part. It could be that at high IAA concentrations, the elevated level of auxin by NPA may be supraoptimal for growth while it is still below optimal level for ethylene production. This may explain the growth inhibition by NPA reported by Knauth and Klämbt (1990). Kang *et al.* (1992) also reported growth inhibition by NPA in *Ranunculus* petioles where auxin does-response curve did not have a biphasic shape, and implied that auxin transport is linked with auxin action on growth by a common system involving the auxin efflux carrier complex.

Fig. 4 illustrates dose-response data for NPA effect on ethylene production in the presence of either IAA or 1-NAA. Since 1-NAA is known to bind to auxin receptors with high affinity (Ray *et al.*, 1977) and is also a substrate of the polar auxin transport system (Hertel and Flory, 1968), NPA effect on ethylene production induced by IAA and 1-NAA was compared. Interestingly, NPA had no effect on 1-NAA induced ethylene production while it strongly stimulated ethylene production by IAA with an optimum at 10^{-5} M NPA. Preliminary results also indicated lack of NPA effect on ethylene production induced by other synthetic auxins (data not shown).

Using thin (1 mm) slices of the coleoptile tissue incubated in a medium containing ^{3}H-IAA or ^{14}C-NAA, we compared NPA effects on net uptake of the two auxins. NPA strongly stimulated IAA uptake by 4-5 fold while only slight increase in 1-NAA uptake was brought about by the same concentration of NPA (Fig. 5). This could explain, in part, the differential effect of NPA on ethylene production observed (Fig. 4). However, the possibility that NPA could affect auxin metabolism which in turn changes free auxin level in the cell cannot be ruled out. In pea stem sections, NPA inhibited IAA oxidation while it stimulated IAA uptake and conjugation (Lee, 1981). However, NPA had no effect on IAA induced ethylene production in that tissues (Lee, 1981), indicating that limiting factors for ethylene production may vary depending on the tissue type. NPA and other transport inhibitors are also known to have no significant effect on ethylene production in roots of intact pea seedlings (Gaither, 1975). In corn coleoptile segments, preliminary results indicate

that the increased intracellular IAA by NPA resulted in the stimulation of IAA conjugation but IAA oxidation was not changed by NPA treatment (data not shown). Intervening role of IAA oxidation in NPA effect on ethylene production is doubtful.

The result presented in Fig. 5 indicating that net uptake of 1-NAA was only slightly increased by NPA compared with IAA uptake although the polar transport of two auxins showed same velocity (Hertel and Flory, 1968) and was equally inhibited by NPA cannot be explained at present. Differential pH optima for uptake may be one possible explanation to be tested.

From our results obtained in the present work, it is suggested that (1) the capacity of auxin transport may be a limiting factor for ethylene production in corn coleoptile tissue as inhibition of auxin efflux by NPA leads to a drastic increase in ethylene production, (2) a positive correlation between differential effects of NPA on intracelluar auxin uptaken and on the ethylene production (Figs. 4 and 5) supports the idea that the primary site of auxin action is an intracellular site (Vesper and Kuss, 1990).

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적 요

옥수수(*Zea mays* L.) 자엽초 조직에서 다양한 오옥신 유도체에 의한 에틸렌 생성을 측정, 비교하였다. 합성 오옥신인 1-NAA와 2,4-D의 경우 에틸렌 생성을 유발하는 효과는 자연 오옥신인 IAA와 비슷하거나 더 높은 양상을 보였으나, 반면 그 유도체인 2-NAA와 2,6-D는 활성이 없었다. 이처럼 생장 촉진효과가 없는 것으로 알려진 오옥신 유도체들이 에틸렌 생성을 유발하는 효과 역시 없다는 사실은 두 가지 생리과정에서 오옥신의 공통적인 작용부위가 있음을 시사한다. 한편 강력한 오옥신 이동 억제제인 NPA의 처리는 IAA 유발 에틸렌 생성을 현저하게 증가시켰다. 그러나 NPA는 1-NAA 유발 에틸렌 생성에는 아무런 효과가 없었다. 1 mm 조직 박편을 이용하여 ^{3}H-IAA와 ^{14}C-NAA 축적을 측정한 실험 결과 세포내 축적된 오옥신의 양과 에틸렌 생성이 밀접한 연관성이 있는 것으로 나타났다. 이러한 실험 결과는 오옥신의 일차적인 작용부위가 세포내에 있을 가능성을 시사한다.

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