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# Specific Inhibition of Polar Auxin Transport by *n*-Octanol in Maize Coleoptiles

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# 옥수수 (*Zea mays* L.) 자엽초 조직 절편에서 *n*-Octanol에 의한 옥신 극성 이동 억제

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**ABSTRACT**

Both polar and gravity-induced lateral transport of auxin was markedly reduced in corn coleoptile segments by octanol treatment. Octanol enhanced net auxin uptake without affecting that of benzoic acid, suggesting that the effect did not result from a nonspecific action on general membrane permeability. Since naphthylphthalamic acid (NPA) action on both transport and net uptake of auxin was substantially decreased in the presence of octanol, a specific interaction of octanol with the NPA site (efflux carrier) can be postulated. Studies on *in vitro* binding of NPA to membrane vesicles indicated that octanol did not interfere with NPA binding. When basipetal transport of auxin was impared by plasmolysis, octanol still inhibited auxin transport in the plasmolyzed tissues. The results ruled out the possibility of octanol acting at the plasmodesmata. Kinetic analysis of growth indicated that IAA-sustained growth was rapidly blocked by octanol implicating a common system by which auxin transport is linked to auxin action. Possible mechanisms for octanol action will be discussed.

## INTRODUCTION

In contrast with the old idea of symplastic pathway of auxin transport (see review of Rubery, 1987), current models for auxin tansport support the view that auxin moves across the plasmalemma with sequential uptake and efflux steps. Two carrier systems in the membrane which have been extensively studied employing *in vitro* experiments (Sabater and Rubery, 1987) provide the basis for the saturability, specificity and polarity found in *in vivo* auxin transport studies (Goldsmith, 1977). The chemiosmotic hypothesis (Rubery, 1987) postulates transmembrane pH and electrical gradient as driving forces of auxin transport.

The relatively high membrane permeability of auxin molecules, and the lack of polarity and selectivity of plasmodesmata (Robards and Lucas, 1990) seem to rule out the possibility of symplastic auxin transport. In fact, attentions on the symplastic pathway for ions and metabolites were made under the circumstances where apoplastic barriers occur (Robards and Lucas, 1990). Nevertheless, progress in studies on the plasmodesmata have focussed on the regulatory role for cell-to-cell communications. Role of plasmodesmata as a main channel of gibberellin transport in the regulation of morphogenesis in antheridal cells of *Chara vulgaris* L. was recently demonstrated

(Kwiatkowska, 1991). Functional studies pointed to the physiological analogies between plasmodesmata and animal gap junctions in spite of the large morphological differences (Robards and Lucas, 1990). Their molecular size exclusion limits (Terry and Robards, 1987), electrical and chemical coupling, and regulation by intracellular factors such as pH, $Ca^{2+}$ and $IP_3$ (Baron-Epel *et al.*, 1988; Tucker, 1988) were strikingly simillar. Moreover, immunological evidences suggested a homologous gap junction polypeptide in plant cells, though yet to be definitely localized to plasmodesmatal structures (Meiners and Schindler, 1987). Although plasmodesmata are complex transwall membranous structures, molucular model suggested that intercellular transport must occur via channels in the cytoplasmic sleeve (Robards and Lucas, 1990). Regulation of voltage-gated channels in the plasmodesmata through which hormones and ions are transported in response to certain stimuli was postulated by Bandurski *et al.* (1990). Gravistimulated lateral transport of auxin could be explainable by this hypothesis.

In view of the analogies between plasmodesmata and gap junctions, role of plasmodesmata on auxin transport was reinvestigated by using octanol, a general anaesthetic agents which block electrical and chemical coupling through gap junction (Spray *et al.*, 1985). We also reexamined the effect of plasmolysis on polar auxin transport to test the possiblity of symplastic pathway for auxin transport.

## MATERIALS AND METHOD

**Plant material.** Presoaked corn (*Zea mays* L. var. Merit) seeds were planted on wet paper towels in plastic trays and placed them vertically in dark at 28℃ with 100% relative humidity. When the seedlings were 3-4 days old, subapical coleoptile segments 3-10 mm in length were cut with a double blade cutter and primary leaves removed. The segments were used for all transport and growth experiments. For NPA binding work, entire coleoptile tissue excluding the primary leaves were used.

**Chemicals and radiochemicals.** [5-$^3$H]-IAA (28 Ci/mmole) and [2,3,4,5-$^3$H]-NPA (55 Ci/mmole) were obtained from CEA (Gif-sur-Yvette, France). $^{14}$C-benzoic acid (16 Ci/mole) was purchased from Dupont.

**Auxin transport tests.** Auxin transport tests were carried out at 28℃ under dim green light. Agar blocks, 3 mm×3 mm×1 mm (1.5% agar buffered with 50 mM sodium phosphate at pH 6.8), containing 38 nM $^3$H-IAA were used as donors. Receiver blocks contained plain buffered agar and test chemicals where indicated. Individual segments were placed vertically, the basal end down, between donor (apical end) and receiver (basal end) blocks. At the end of a transport period, the radioactivity in the receiver blocks was counted with a liquid scintillation spectrometer.

**Auxin uptake studies.** Net uptake of auxin was measured as described previously by Yoon and Kang (1992). Subapical corn coleoptile tissues were cut into 1 mm slices with a multibladed cutter. Twenty slices were incubated with 1 m*l* of 10 mM sodium phosphate buffer at pH 5 containing 8.9 nM $^3$H-IAA and other chemicals in a 20 m*l* vial. At the end of a 30 min uptake period, the medium was rapidlly removed and the slices were washed twice with cold buffer under reduced pressure. Ten slices were transferred to a scintillation vial for counting radioactivity.

***In vitro* binding of NPA.** All procedure were carried out at 4℃ according to Kang (1986) with slight modifications. Coleoptiles were chopped and ground in the extraction medium (50 mM Tris-acetate at pH 8, 0.25 M sucrose, 1 mM EDTA, 0.1 mM $MgCl_2$, 1 mM DTE). The crude homogenate was squeezed through a nylon cloth and centrifuged for 10 min at 4,300×g. The resulting supernatant was centriguged at a high speed (200,000 g) for 20 min in a Beckman Ti 45 rotor. The supernatants were discarded, and the pellets resuspended in binding assay medium (10 mM sodium citrate at pH 5.5, 0.25 M sucrose, 0.5 mM $MgCl_2$, 5 mM $MgSO_4$) with a glass homogenizer. $^3$H-NPA (0.1 nM) was added to the resuspended particles with or without various concentration of cold NPA. The mixtures were centrifuged for 20 min at 200,000 g in a Beckman Ti 75 rotor. All the supernatants were decanted and 1 m*l* of methanol was added to each tube. After 1 h extraction of radioactivity in the pellet, the methanol was transferred to a scintilation vial for counting radioactivity.

**Short-term growth kinetics.** Growth rates were measured with a linear-displacement transducer (Series 605-2.5, Erichsen, Wuppertal, Germany) using single segments as described by Kutschera and Schopfer (1985). Individual, 10 mm subapical coleoptile segments placed in the transducer were incubated with 7 m*l* of distilled water at 27℃ with constant airation. After a 60-90 min incubation at which the endogenous growth rates of the segments reached to a steady state, test chemicals were added to the medium. Both growth and its rate were recorded with a two channel chart recorder set to 6 mm/h.

Table 1. Effect of n-octanol on basipetal polarity of auxin transport in corn coleoptile segments

| Pretreatment[a] | Auxin transport (cpm)[b] | | Polar ratio (basipetal/ acropetal) |
|---|---|---|---|
| | Basipetal | Acropetal | |
| None | 3,368 | 290 | 11.6 |
| *n*-Octanol | 1,372 | 306 | 4.5 |

[a]Coleoptile segments (10 mm) were preincubated for 90 min in the presence or absence of 1 mM n-octanol, and cut into 5 mm segments for subsequent auxin transport test. [b]Auxin transport test was carried out in the dim green light at 28°C for 2 h with 100% humidity. In acropetal transport, donors were placed at the basal end of the segments.

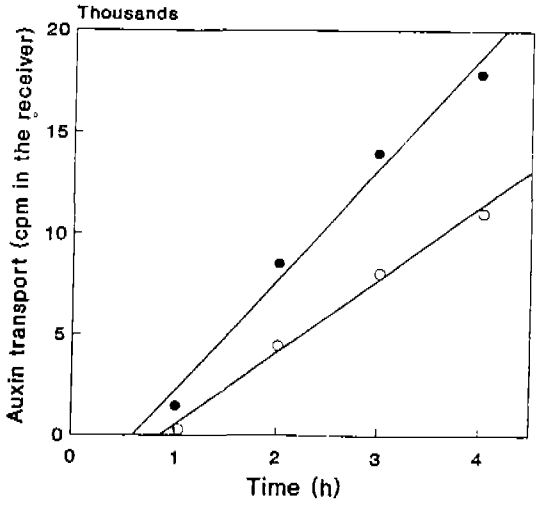


Fig. 1. Time course of auxin transport in corn coleoptile segments with (○) or without (●) n-octanol pretreatment. Prior to auxin transport test, coleoptile segments were preincubated for 90 min in the presence or absence of n-octanol at 1 mM. At the end of each time interval as indicated, receiver blocks were removed for counting radioactivity.

## RESULTS

**Effect of n-octanol on auxin transport.** Basipetal transport of labelled auxin in corn coleoptile segments was severely blocked by octanol pretreatment with acropetal transport virtually unaffected leading to a decrease in the polar ratio (basipetal/acropetal transport) of the tissue (Table 1). Kinetic data indicated that octanol reduced the transport capacity (Fig. 1). Pattern of auxin transport inhibition by octanol appears linear at the concentration range from 0.1 to 1 mM (Fig. 2). Studies on the effects of various primary alcohols on auxin transport indicated a strong correlation between alkyl chain length and transport inhibition (Table 2), implicating a mode of action involving their lipid solubility. However, the result that octanol did not affect passive uptake of a weak acid ($^{14}$C-benzoic acid), implied that the effect may not be one related to general membrane permeability (Table 3). This is also supported by the finding as presented in Table 1 that octanol had no effect on the acropetal transport of auxin which is strictly by diffusion (Goldsmith, 1977). Rather, a specific inhibition of polar auxin transport by octanol could be postulated since octanol treatment led to a strong promotion of net uptake of labelled auxin by thin (1 mm) sections of the coleoptile (Table 3). The relationship between net uptake and transport of auxin in corn coleoptile tissue was well established; either saturating efflux (Edwards and Goldsmith, 1980) or blocking it with transport inhibitors (Sussman and Goldsmith, 1981) lead to increased net uptake of auxin by the tissue. NPA is known to inhibit polar transport of auxin by spe-

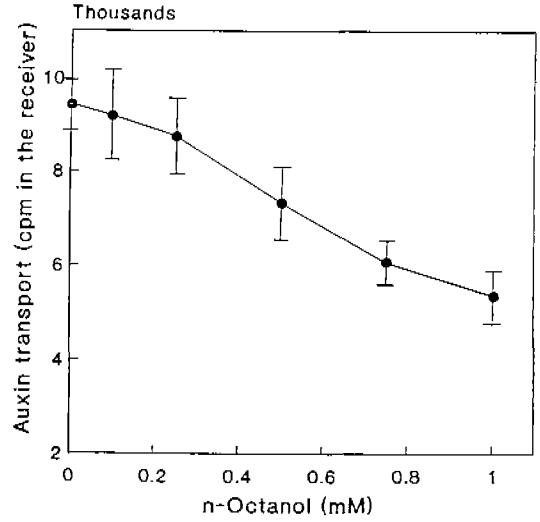


Fig. 2. Effect of various concentrations of n-octanol on auxin transport in corn coleoptile segments. Subapical segments (1 cm) were preincubated for 90 min with n-octanol at indicated concentrations and cut into 5 mm sections for subsequent auxin transport test. After 2 h transport period, radioactivity in the receivers was counted. Bars indicate standard deviation from three, duplicate experiments.

Table 2. Correlation between alkyl chain length of various primary and secondary alcohols and transport inhibition in corn coleoptile segments

| Alkyl chain length | Transport inhibition (%)[a] |
|---|---|
| $C_4$ (*n*-Butanol) | 89± 5[b] |
| $C_5$ (*n*-Pentanol) | 90± 5 |
| $C_6$ (*n*-Hexanol) | 89± 4 |
| $C_7$ (*n*-Heptanol) | 77± 8 |
| $C_8$ (*n*-Octanol) | 35± 4 |
| $C_8$ (2-Octanol) | 56± 3 |
| $C_{10}$ (*n*-Decanol) | 21± 2 |

[a]Coleoptile segments (10 mm) were preincubated for 90 min at 28℃ with or without indicated alcohols (1 mM) and cut into 5 mm sections for subsequent auxin transport test. Transport test was carried out in the dim green light at 28℃ for 2 h. Values are expressed as percent of control. [b]Average values± standard deviation from two experiments with duplicates.

Table 3. Comparison of net uptake of $^3$H-IAA and $^{14}$C-benzoic acid (BA) by 1 mm corn coleoptile sections

| Compounds (Treatment) | Net uptake (cpm in the tissue)[a] | |
|---|---|---|
| | Control | (+) n-Octanol[b] |
| $^3$H-IAA (− NPA)[c] | 3,752 | 6,861 |
| (+ NPA) | 15,592 | 10,508 |
| $^{14}$C-BA (− NPA) | 5,614 | 5,026 |
| (+ NPA) | 4,786 | 5,165 |

[a]Thirty, 1 mm corn coleoptile sections were incubated with $^3$H-IAA or $^{14}$C-benzoic acid in buffer (50 mM, potassium phosphate buffer, pH 6.8) for 30 min and the radioactivity in the tissues were counted. [b,c]Both NPA (10 μM) and n-octanol (1 mM) were added in the incubation medium. Values are representative data from three experiments with duplicates.

cifically blocking the efflux carrier on the plasmalemma and hence increases net uptake of auxin both *in vivo* (Sussman and Goldsmith, 1981) and *in vitro* (Hertel *et al.*, 1983). Studies on *in vitro* binding of labelled NPA to membrane vesicles isolated from the coleoptile tissues indicated that octanol had a neglegable effect on the NPA binding activity (Table 4). Since octanol was a poor competitor for the NPA binding site, a possible mode of its action as a phytotropin was ruled out (Katekar and Geissler, 1980). In addition, unlike NPA, octanol applied in

Table 4. Effect of n-octanol on the specific binding of $^3$H-NPA to membranes isolated from corn coleoptile segments

| Treatment[a] | Specific binding (cpm)[b] |
|---|---|
| None | 566 |
| *n*-Octanol 0.5 mM | 537 |
| *n*-Octanol 1 mM | 541 |

[a]n-Octanol was added to the membrane suspension. [b]Specific binding denotes the difference in $^3$H-NPA binding (cpm) between plus and minus 10 μM cold NPA. Values are representative data from four experiments with duplicates.

Table 5. NPA action on auxin transport in the presence or absence of n-octanol

| Treatment[a] | Transport (cpm)[b] | | |
|---|---|---|---|
| | (−) NPA | (+) NPA | % Inhibition[c] |
| None | 3,368 | 1,010 | 70 |
| *n*-Octanol | 1,372 | 669 | 51 |

[a]For transport test, tissues were pretreated with n-octanol (1 mM) for 90 min. [b]Auxin transport test was carried out for 2 h in the presence or absence of 1 μM NPA in receivers. [c]Values are expressed as percent inhibition of auxin transport by NPA. Representative data from two experiments with duplicates.

the receiver or donor blocks had no effect on the transport of auxin (data not shown). However, since NPA effect on both transport (Table 5) and net uptake of auxin (Table 3) was substantially decreased in the presence of octanol, it is possible that octanol could interact specifically with NPA site (i.e. efflux carrier). Lateral (downward) transport of labelled auxin in gravistimulated coleoptile segments was also inhibited by octanol pretreatment (Table 6). Delayed gravitropic responses were observed in those tissues (data not shown).

**Kinetic analysis of growth.** Octanol treatment was found to rapidly abolish the IAA-induced growth (Fig. 3). Octanol is known as a general uncoupling agent of gap junction channel in both excitable (such as liver or embryonic cells) and inexcitable (heart or smooth muscle, neuron) tissues (Spray *et al.*, 1985; Duncan *et al.*, 1988; Wood *et al.*, 1990). Electrical and chemical coupling is blocked by octanol concentrations at 0.1 to 1 mM depending on the tissue type. Since functional similarity had

Table 6. Lateral transport of labelled auxin in corn coleoptile segments

| Pretreatment[a] | Ratio of radioactivity (cpm) of the lower to upper halves of tissue[b] |
|---|---|
| None | 1.42± 0.04[c] |
| *n*-Octanol 0.5 mM | 1.30± 0.02 |
| *n*-Octanol 1.0 mM | 1.12± 0.08 |

[a]Corn coleoptile segments (10 mm) were preincubated for 90 min at 28℃ in the dark with shaking. [b]Vertically oriented coleoptile segments were preloaded with donor ($^3$H-IAA) for 1 h. After preloading the segments were horizontally placed for another 1 h. Radioactivity transported laterally to the each side (upper and lower) of the tissuse was measured. [c]Average values± standard deviation from three experiments with duplicates.

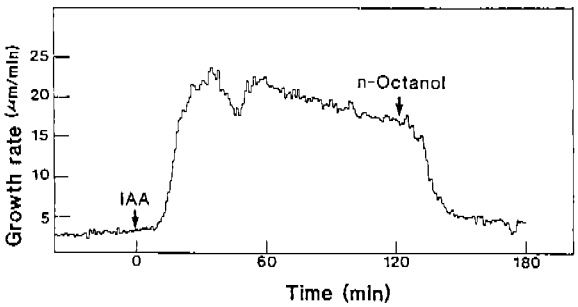


Fig. 3. Effect of n-octanol on IAA-induced elongation in corn coleoptile segment. At time zero, which was actually 90 min after cutting the segment, 10 μM IAA was added, and 1 mM n-octanol at time 120 min.

been made between animal gap junctions and plant plasmodesmata (Meiners *et al.*, 1988) and there are immunological evidences that a gap junctional polypeptide may be a central element of plasmodemata (Meiners and Schindler, 1987), we tested the possibility that the action site of octanol on auxin transport could be plasmodesmata.

**Auxin transport in plasmolyzed tissue.** Auxin transport should be blocked by plasmolysis if transport occurred via symplastic pathway because plasmodesmatal connection is known to be disrupted under the condition. This is the case when the corn coleoptile cells were severly plasmolyzed in 0.5 M mannitol for 30 min. Under microscopic observations (×400), most cells of the tissue showed complete plasmolysis with the plasma membrane retracted from the cell wall at one or both ends with few protoplasmic connections to the wall remaining. The fact that basipetal transport of auxin was impared, although not completely, in those plasmolyzed tissue (Fig. 4) was in agreement with that reported for *Avena* coleoptiles (Cande and Ray, 1976), suggesting that movement through the plasmodesmata accounts for a significant, if not all, portion of the polar auxin transport.

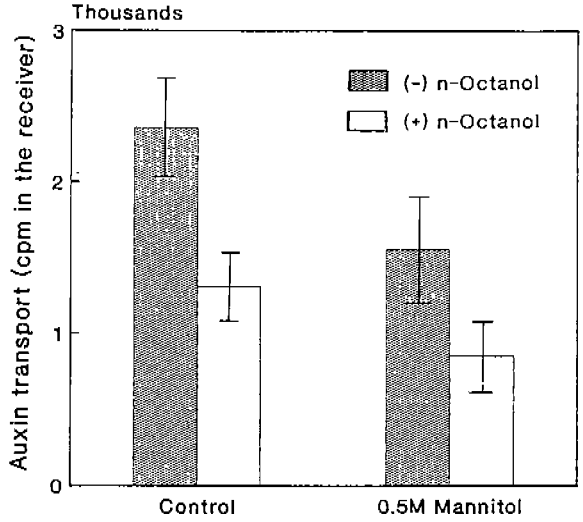


Fig. 4. Effect of plasmolysis on auxin transport in corn coleoptile tissue in the presence or absence of n-octanol. For plasmolysis, subapical coleoptile segments (1 cm) were preincubated in 0.5 M mannitol for 30 min with or without 1 mM n-octanol. Controls were preincubated with distilled water for 30 min. Under the light microscopy (×400), severe plasmolysis were observed. After 1 h transport, radioactivity in the receiver was counted. Bars indicate standard deviation from three experiments with duplicate data.

If octanol was blocking the symplastic pathway through plasmodesmata, one would expect that the inhibitory effect of octanol on transport dissapear in the plasmolyzed cells. The inhibitory rate of transport by octanol was, however, almost identical with or without plasmolysis (Fig. 4). The fact that octanol still inhibited auxin transport in plasmoyzed tissues indicates that the alcohol must be acting at some site other than (or in addition to) the plasmodesmata.

## DISCUSSION

The inhibition of auxin transport by octanol in corn coleoptile segments seems to be specific since our results

indicated that (1) the polarity of both basipetal and lateral transport of the tissue were markedly reduced by octanol (Tables 1, 6), (2) the differential effects of octanol on the net uptake of $^3$H-IAA and $^{14}$C-benzoic acid (Table 3) were observed, and (3) octanol reduced the activity of NPA, a specific inhibitor of the auxin efflux carrier (Tables 3 and 5). Our present results strongly suggest a specific interaction of octanol with the NPA site. When discussing mechanisms for octanol action, questions as to whether it inhibits auxin transport by blocking the plasmodesmata channel may be raised. Our results (Fig. 4) indicated that the inhibitory effect of octanol on auxin transport was still apparent in tissues where symplastic connections were disrupted, suggesting that octanol might not act on the plasmodemata. The auxin efflux carriers were found to preferentially localized at the basal end of a cell instead on the plasmodesmatal region (Jacobs and Short, 1985). However, the possibility of uncoupling of plasmodesmatal channel by octanol as in gap junctions of the animal system can not be totally excluded since channels in the membranous part of the plasmodesmata could be functionally intact even under the conditions where symplastic continuity is disrupted.

Although the potency of transport inhibition of octanol is related to its lipid solubility (Table 2), a nonspecific effect on general membrane permeability and/or transmembrane pH gradient can be ruled out since octanol was found to have no effect on passive diffusion of the non-auxin, benzoic acid (Table 3) having a pK value similar to that of IAA. The report that passive $^{86}Rb^+$ leak from sealed synaptic vesicles was only significant at high concentrations of octanol (Wood *et al.*, 1990) was in line with these results. It is also unlikely that the octanol effects were of secondary nature due to metabolic damages of the cell.

Possible mechanisms for the octanol action on auxin transport could be postulated. First, the interface between membrane lipid and transport carrier protein may be perturbed by octanol. Some membrane-bound proteins require the presence of tightly bound lipid to stabilize them in their active form. General anaestetics, including octanol, could disrupt the structural role of lipids. They could also cause membrane expansion by increasing the disorder of the fatty acid which lead to impaired membrane function (Richards, 1976). Modification of auxin efflux carrier by diethyl ether, another anaesthetic agent, was implicated as a possible result of interaction between transport carrier and membrane lipids (Kang, 1986). Phospholipase sensitivity was reported for NAA binding sites (Ray and Dohrmann, 1977).

Although *in vivo* results suggested a interaction with the NPA site, competition at ligand site can be ruled out from the *in vitro* NPA binding study (Table 4). But it should be noted that the octanol action could be temperature dependent (Robards *et al.*, 1978).

A second possiblity involves functional uncoupling of auxin transport carrier by octanol. Since our results indicated a rapid and complete blockage of IAA-induced growth by octanol, uncoupling at a site common to both auxin transport and action could be postulated. Auxin efflux carrier was proposed to be responsible for the auxin action on growth through a mechanism involving calcium channel gating (Hertel, 1983). Kang *et al.* (1992) reported growth inhibition by NPA in *Ranunculus* petioles implying auxin efflux carrier as a common system for auxin transport and its action on growth. A tight link between calcium and auxin fluxes in both basipetal (de La Funte *et al.*, 1985) and lateral (Gross and Sauter, 1987) transport was demonstrated. Calcium channels in the plasmalemma and tonoplast were identified recently (Schroeder and Thuleau, 1991). Octanol-like molecules could block calcium channel gating as they do on Na channel (Haydon and Urban, 1983) or acetylcholine receptor channel (Murrell *et al.*, 1991) leading consequently to a functionally impaired efflux carrier. Inhibition of the calcium influx by octanol in isolated rat heart cells (Haworth *et al.*, 1989) raises this possibility. It is also possible that changes in the transmembrane electical potential by octanol due to its effect on the transmembrane ion current was responsible for the transport inhibition. *In vitro* studies indicated that uptake carriers are electrogenic (Sabater and Rubery, 1987).

Concerning the symplastic pathway for auxin transport, our results (Table 7) suggest that a significant portion of the polarity of auxin transport might be represented by this pathway. Considering the possibility that transport function of membraneous channel in the plasmodesmata still remained intact upon complete plasmolysis, it should be pointed out that auxin transport through plasmodesmatal channel might have been underestimated before (Cande and Ray, 1976; Drake and Carr, 1978). Movement of auxin from stele to cortex in maize mesocotyl through voltage-gated channels in the plasmodesmata as transducers of gravitropic stimulus was postulated to have a regulatory role on the gravitropism (Bandurski*et al.*, 1990). Tissue printing and autoradiography in maize coleoptile

segments revealed that auxin polar transport occured primarily in association with vascular tissue (Cha *et al.*, 1991). The results implied that interchange of auxin between vascular tissue and epidermal cells sensitive to IAA could occur possibly via plasmodesmatal transport pathway.

## ACKNOWLEDGEMENT

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## 적 요

옥수수 자엽초 조직 절편에서 옥신 극성 이동과 측면 이동이 octanol 처리에 의해 현저히 억제되었다. 자엽초 조직 박편을 이용한 실험 결과 octanol이 옥신 축적을 현저하게 증가시킨다는 결과를 얻었다. 반면 IAA와 비슷한 약산이면서도 특정 운반체가 관여하지 않는 것으로 알려진 benzoic acid의 축적에는 아무런 영향도 주지 않았는데 이는 octanol의 효과가 단순히 세포막에 비특이적으로 작용한 결과가 아님을 시사한다. 한편 octanol이 있는 조건에서 옥신 이동과 옥신 축적에 대한 naphtylphthalamic acid (NPA)의 효과가 감소한 실험결과는 octanol이 NPA 작용부위(즉, 옥신 efflux carrier)에 특이하게 작용할 가능성을 시사한다. 그러나 octanol은 *in vitro*에서 세포막의 NPA binding에 거의 영향을 주지않은 결과로 보아 octanol이 NPA 수용체에 결합하여 옥신 이동을 억제할 가능성은 배제된다. 원형질 분리를 유도한 조직에서 옥신 극성이동능이 현저하게 감소한 결과로 보아 옥수수 자엽초 조직 절편에서 옥신 극성 이동량의 일부분은 원형질연락사 경로를 이용한다고 추측된다. 그러나 그런 조직에서도 octanol의 옥신 이동 억제효과는 여전히 나타났으므로 octanol의 작용부위는 원형질연락사는 아니라고 생각된다. 한편 octanol이 옥신에 의한 생장을 빠른 시간내에 억제한다는 결과를 얻었는데 이는 옥신 이동과 작용에 공통적인 요소가 있음을 시사한다.

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