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THE EFFECT OF ABSCISIC ACID ON THE AUXIN TRANSPORT IN MUNGBEAN HYPOCOTYL SEGMENTS

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Abstract

The basipetal transport of IAA (indoleacetic acid-2-^{14}C) was studied in mungbean (*Vigna radicata* L.) hypocotyl segments using the method of donor-tissue-receiver system. ABA (abscisic acid) inhibited IAA basipetal transport. Kinetic analysis showed that ABA at 10^{-5} M reduced the transport intensity by about 51% and transport density by 68%. The velocity and total auxin uptake from donor blocks were not reduced by ABA. Chromatographic analysis of the radioactivity held in the tissue revealed that ABA did not affect the metabolism of IAA. However, ABA stimulated the decarboxylation of IAA-1-^{14}C but did not affect the metabolism of the IAA at the second carbon. Moreover, ABA inhibited IAA-2-^{14}C binding to a particulate cell fraction presumbably plasma membrane visicles. It was suggested that the inhibition of IAA transport by ABA was mainly due to the stimulation of IAA decarboxylation and the interaction of ABA and the IAA transport carrier system in the membrane.

Introduction

Endogenous hormones may work together to control plant development is widely accepted among plant scientists. Indoleacetic acid (IAA) and abscisic acid (ABA) have been found to occur in many of the plant species examined, and they appear to be closely associated with gibberellins (GA) in control of growth and dormancy in plants (Wareing and Saunders, 1971). At onset of dormancy there is a high concentration of ABA in apical buds, while promoting hormones such as GA and IAA occur in high concentrations in the rapidly expanding buds and young shoots. The causes of the changes in IAA level at the stage of dormancy have not been elucidated. One possible contributing factors could be an involvement of ABA and GA in affecting the rate of IAA translocation.

ABA has been reported to regulate abscission of cotton fruit (Addicott

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and Lyon, 1969) and *Lupinus* fruit (Porter and Van Steveninck, 1966). Bottgen (1970a, b) demonstrated the abscission-speeding action of ABA on the explant of *Coleus.* The endogenous auxin IAA is generally believed as the controlling agent preventing abscission (Jacobs, 1962, 1962, 1968). IAA produced in the leaf blade moves down into the petiole to inhibit abscission. Thus, the ability of the petiole to transport IAA acts as a control on abscission; as the leaf ages, its ability to transport IAA steadily declines (Venn, 1967). Hence an understanding of the regulation on IAA transport in aging petioles would be helpful in elucidating the aging process.

While ABA has been shown to affect IAA transport in plant segments (Basler, 1971; Chang and Jacobs, 1973; Naqvi and Engvild, 1974), other has reported that ABA has no effect on the transport of IAA (Osborne and Mullins, 1969). The present studies, therefore, was undertaken to re-examine the effect of ABA on the transport of IAA. In order to facilitate the studies of IAA transport kinetics, hypocotyl sections and the donor block-tissue-receiver block system have been employed.

Material and Methods

Plant material

Mungbean seedlings (*Vigna radicata* L.) were grown in a mixture of equal amount of sand and vermiculate moistened with tap water. The seedlings were grown in a growth chamber at room temperature in the dark. Plants were harvested for experiments 4 days after sowing when they had reached a height of about 6 cm with a well-developed hypocotyl.

Chemicals

The indole-3-(acetic acid-2-^{14}C) (S. A. 55 mC/mmole) and indole-3-(acetic acid-1-^{14}C) (S. A. 52 mC/mmole) were purchased from Radiochemical Center, Amersham, England. RS-abscisic acid was generously provided by Hoffmann-LaRoche Inc., Nutley, New Jersey, U. S. A.

Preparation of agar block for donor-tissue-receiver transport system

Bacto Agar at a concentration of 1.5% was added to a 10^{-3} M phosphate-citrate buffer, pH 4.6. For the preparation of donor blocks, the agar was melted, and after the temperature dropped to about 60°C, the radioactive compound was added and mixed thoroughly. Immediately afterwards, the agar was poured into ice cooled Petri dishes, and the agar was cut into discs with a cork borer. Receiver blocks were prepared in the similar manner except that the radioactive compounds as indicated. The agar discs had a diameter of 11 mm and 2 mm thickness. The volume of the disc was 1.90 ml.

Transport tests

A 5-mm section was cut off from each seedling at a distance of 2.0 cm below the hypocotyl hook. Eight pieces of these sections were then placed on a receiver agar block mounting on a microscope glass slide. The donor block, placed on another glass slide, was then pressed down against the receiver agar block until all sections made good contact with both blocks. Each assembly was subsequently placed in a Petri dish whose bottom was covered with a moistened filter paper to keep the relative humidity high. At given time intervals, the assembly was transferred to a counting vial containing 1.5 ml methanol. Radioactivity was extracted from the agar and the tissue for 24 hr. Then, 15 ml of liquid scintillation cocktail (PPO, 8 g/l; naphthalene, 100 g/l; and *p*-dioxane) was added to each vial, and radioactivity was measured with a liquid scintillation counter. Experimental results were presented as a mean of triplicates with each experiment repeated at least twice.

Decarboxylation experiments

IAA decarboxylation activity was assayed by a modified technique of Kerstelter and Keitt (1966). The reaction was carried out in a scintillation counting vial which was stoppered with a rubber cap. A polyvial (2 ml in volume) containing reaction mixture was suspended with a Scotch tape attached to the cap. A volume of 0.2 ml of 20% KOH was added to the bottom of the scintillation vial for trapping $^{14}CO_2$ evolved.

Eight 5-mm hypocotyl segments were placed in the polyvial, and the reaction mixture containing 0.5 ml of 10^{-3} M citrate-phosphate buffer (pH 4), 0.1 ml of 2,4-dichlorophenol (0.5 mmoles) and 0.1 ml of $MnCl_2$ (2 mmoles) was added. The reaction was started by adding the radioactive IAA to the reaction vial. The vail was then tightly capped. After 3 hrs of incubation at 25°C, the reaction was stopped by adding 0.2 ml of 0.1 N HCl. The vial was incubated for another 20 minutes to stop the enzymatic reaction and to trap the $^{14}CO_2$ by KOH. The cap was then removed, the KOH solution was neutralized with HCl, and then 15 ml of liquid scintillation cocktail was added for radioassay.

Chromatography

Paper chromatography was used for the analysis of radioactivity in the tissue. Radioactivity was extracted with absolute methanol for 24 hr at 2°C. An aliquot (0.1 ml) of each extract was then applied to paper strips (Whatman No. 1). Chromatograms were developed in isopropanol-28% ammonia-distilled water, 8:1:1 (v/v), by descending chromatography. Radioactivity of each Rf zone was extracted in a liquid scintillation counting vial containing 1.5 ml

methanol before 3 ml of liquid scintillation cocktail was added for radioactivity determination. The authentic IAA and IAA-2-^{14}C were also chromatographed. IAA was determined by spraying with Salkowski's reagent (Gordon and Weber, 1961).

Preparation of homogenates for IAA-2-^{14}C binding assay

Approximately 30 g of hypocotyl tissue was ground in a mortar and pestle with 50 ml of medium containing 2% sucrose, 50 mM Na_2EDTA, 80 mM Tris-acetate (pH 6.5), 100 mM KCl, 25 mM $MgCl_2$ and 5% polyvinyl pyrrolidone (PVP). Immediately after homogenization, $CaCl_2$ was added to obtain a final concentration of 25 mM. The resulting pH of the homogenate was around 5.5. The homogenate was strained through one layer of nylon cloth, then centrifuged at 800×g for 10 min. The supernatant fraction recovered was subsequently fraction recovered was subsequently centrifuged at 2500×g and 5000×g, respectively, with each centrifugation for 10 min. To 1.8 ml of the supernatant fraction recovered from the series of centrifugations, various amount of ABA or IAA and approximately 5000 dpm of IAA-2-^{14}C were added to make a final volume of 2.0 ml. The mixture was incubated for 5 min at 25°C. The reaction mixture was filtered through a Selutrofilter BA85 (0.45 μm). An aliquot (1.0 ml) of the filtrate and the entire filter paper were transferred respectively to vials containing 3 ml scintillation counting solution, and radioassayed in a liquid scintillation counter.

Results and Discussion

Effect of ABA on the basipetal movement of IAA

Figure 1 shows that ABA at physiological concentrations inhibit the basipetal transport of IAA-2-^{14}C. ABA at a concentration of 10^{-5} M inhibits 22% of IAA transport. Thus ABA at a concentration of 10^{-5} M was used throughout the rest of the study.

Effect of ABA on the parameters of IAA transport

Keldeway (1966) had described the rationale for measuring the parameters of IAA transport. To measure these parameters, radioactive IAA-2-^{14}C was applied in donor blocks to the apical end of hypocotyl section. The radioactivity was collected in the receiver blocks at 30 min intervals. A plot of the amount of radioactivity delivered in the receiver blocks versus time is shown in Figure 2. The calculated parameters for auxin transport in the control and ABA treated sections are shown in Table 1. The velocity of IAA transport was found to be 6.25 mm/hr, which agree with the 6.7 mm/hr found

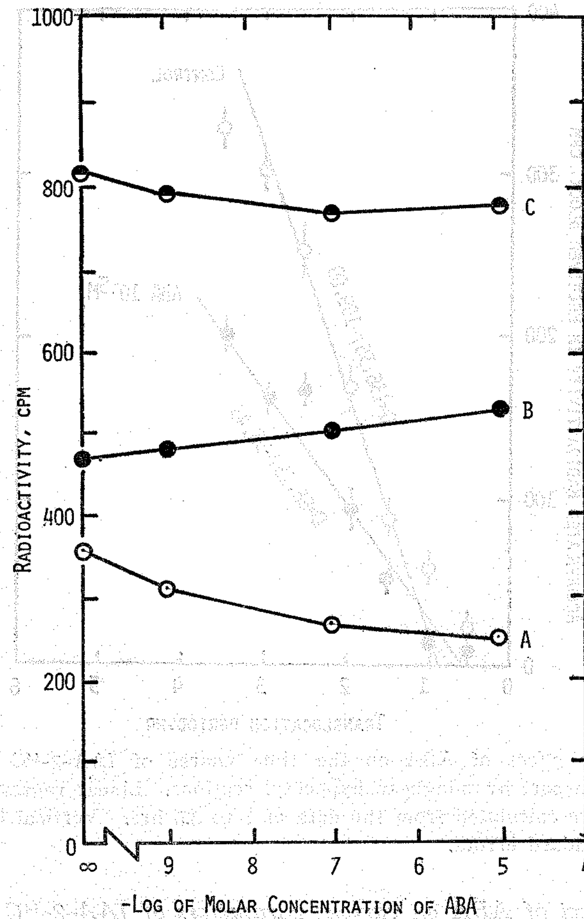

Fig. 1. Effect of ABA on the total IAA uptake by mungbean hypocotyl segments during 3 hr of basipetal transport. Radioactivity in receiver block (A), in tissue segment (B), and the total uptake (C=A+B) are shown. ABA was incorporated in receiver blocks.

by Smith and Jacobs (1969) in bean hypocotyls, and with that of 5.7 mm/hr for bean petiole section found by McCready and Jacobs (1963). ABA at 10^{-5} M reduced the transport intensity by about 51% (136 cpm/hr versus 66 cpm/hr). A reduction in transport intensity could be the result of a low velocity or low density, or both since intensity is equal to the product of the product of density and velocity. Our data show that the velocity was not reduced by ABA, Instead it was increased (Table 1). The density was reduced about 69% (7 cpm/mm versus 22 cpm/mm) by ABA.

On the basis of these data, we conclude that the inhibition of auxin transport by ABA is the results of low transport density, i.e., less transportable auxin is present per unit length of section, but not a result of reduced trans-

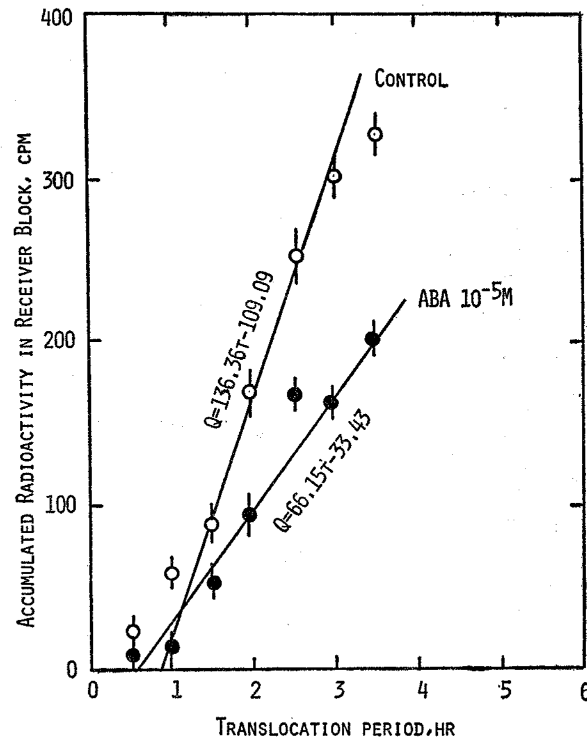

Fig. 2. The effect of ABA on the time course of IAA-2-^{14}C basipetal transport by mungbean hypocotyl sections. Linear regression lines were calculated from the data of 1 to 3.5 hrs. Vertical bars were standard errors.

Table 1. *Effect of ABA on various parameters of IAA-2-^{14}C transport in 5-mm segments of mung bean hypocotyls*

Data were taken from Fig. 2.

Transport parameters	Control	ABA, 10^5-M	% Inhibition
Velocity, mm/hr	6.25	9.89	58.24
Density, cpm/mm	21.82	6.69	69.34
Intensity, cpm/hr	136.38	66.16	51.48

port velocity. A low transport density could be caused by: 1) inhibition of IAA uptake by hypocotyl sections: 2) IAA immobilization by conjugation with other compound or by being bound to macromolecules; 3) IAA decarboxylation in the tissue; 4) inactivation of the polar transport system, IAA might be present in the tissue as a intact and free molecule, but no transport could take place because of lacking active transport sites. Efforts were then made to test the above possibilities.

Uptake of IAA-2-^{14}C by tissue sections

The total uptake of IAA was calculated as the sum of the radioactivity exported from the tissue to the receiver block plus the amount which was hold in the tissue. ABA showed a typical 20% inhibition of IAA transport, but there was no significant difference in total uptake (Fig. 1). Furthermore the difference in transported auxin between control and ABA treated tissue can be accounted for by the extra amount of IAA found in the tissue treated with ABA. The results indicates that ABA inhibits the exist of auxin from the tissue but not the entry into the tissue.

The relationship between auxin accumulation in the tissue and inhibition of transport density by ABA (Table 1) could be interpreted in following ways: 1) ABA stimulated IAA metabolism including decarboxylation, so that only a small amount of IAA-2-^{14}C remained intact and available for transport, while the rest accumlated in the tissue; 2) ABA inhibited auxin transport *per se* and interact with IAA or by disrupting the auxin transport system. The result of this action would be the accumulation of IAA.

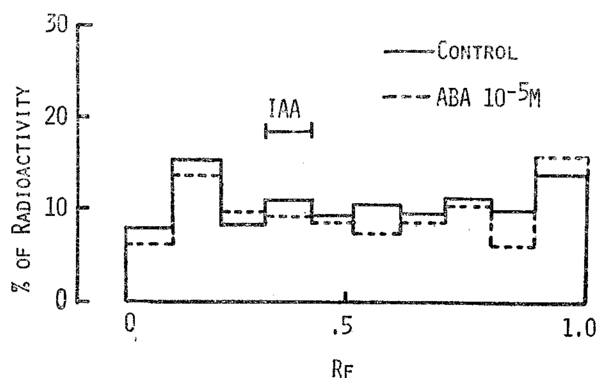

Fig. 3. The distribution of radioactivity on the chromatogram of the methanol extracts from blank tissue and ABA-treated tissue of mungbean hypocotyls.

Metabolism of IAA-2-^{14}C in hypocotyl sections

After 3 hrs of IAA transport, only 11% and 9%, respectively, of the radioactivity in the control and ABA-treated hypocotyl section were chromatographed as IAA. There was another major spot with 17% of the total radioactivity present in the control and 15% in the case of ABA treatment; the Rf value was about 0.20. However, due to the lacking of authentic compounds of IAA metabolism, no attempt was made to identify the compound(s). The insignificant difference between control and ABA treated tissue with respect to the concentration of free IAA was not enough to account for 22% inhibition of

IAA transport. Any change in IAA metabolism is not the cause of ABA-mediated inhibition of IAA transport.

Decarboxylation of IAA-1-^{14}C and IAA-2-^{14}C

Decarboxylation of IAA occurs as a results of photooxidation (Fukuyama and Moyed, 1964) or enzymatic oxidation catalyzed by peroxidase (Hillman and Lang, 1965; Still *et al.*, 1965). Hager and Schmidt (1968) have shown that enzymatic or photooxidation of IAA leads to the formation of 3-methylene-oxidole which inhibits auxin transport in corn coleoptiles. It is possible that ABA might inhibit active transport by speeding up IAA oxidation resulting in an increased in the amount of 3-methyleneoxidole. As shown in Fig. 4 ABA stimulated the rate of decarboxylation but did not affect the metabolism of the IAA at the second carbon. Due to lack of authentic cold and labelled metabolites of IAA decarboxylation, no attempt was made to investigate the behavior of the transport (rate, polarity etc.) of these metabolites. The relationship between promoting effect of ABA on IAA-1-^{14}C decarboxylation and the inhibition of IAA transport remains to be examined.

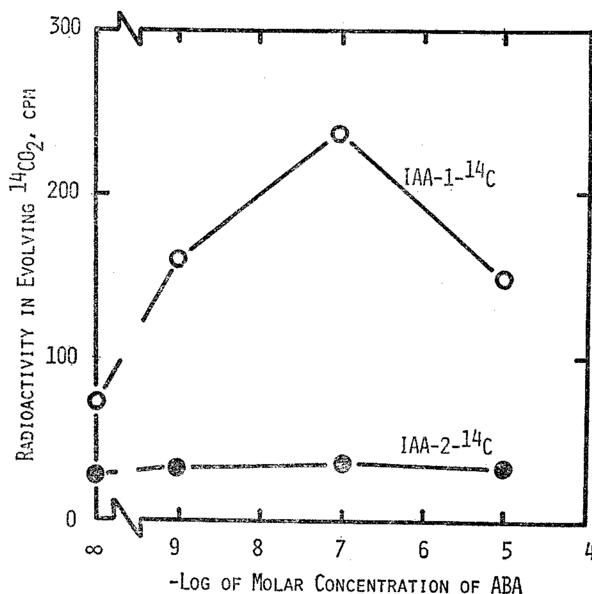

Fig. 4. The effect of ABA on decarboxylation of IAA-1-^{14}C and IAA-2-^{14}C in the segments of mungbean hypocotyls.

In vitro auxin binding to membrane-rich fraction

The polar transport of IAA through tissue probably involves a passive uptake of auxin into the cells and a preferential "secretion" of auxin from

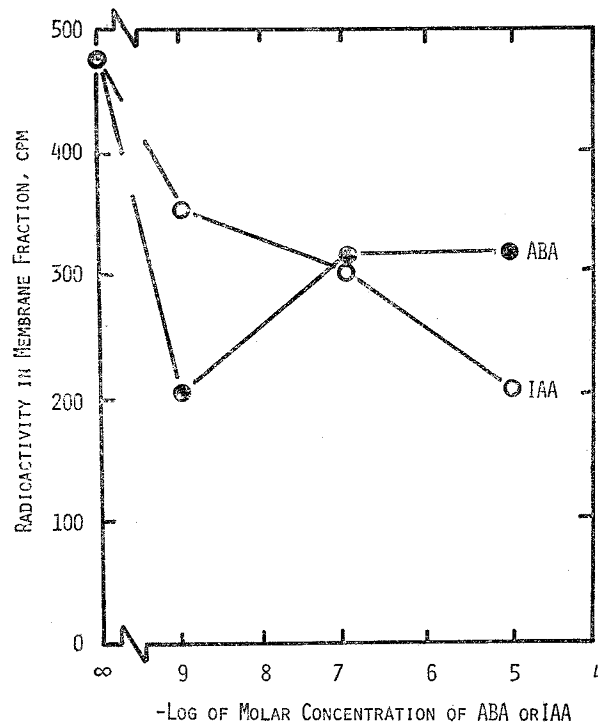

Fig. 5. The effect of ABA and IAA on the binding of IAA-2-^{14}C to the membrane fraction of mungbean hypocotyls.

the basal ends of the cells (Hertel and Leopold, 1963; Hertel and Flory, 1968; Goldsmith and Ray, 1973). Indirect evidence and theoretical arguments suggest that this "secretion" of auxin may depend on a specific auxin carrier associated with the membrane (Hertel and Leopold, 1963; Hertel, *et al.*, 1969). As shown in Fig. 5, both unlabelled IAA and ABA inhibited the binding of IAA-2-^{14}C to the membrane-rich fraction. On the basis of this preliminary binding data, it is likely that ABA may interact with the IAA carrier system in the membrane, thus preventing the transport of IAA across the membrane.

In view of above findings, the ABA-stimulated IAA decarboxylation and inhibiting action on IAA binding to the membrane system are likely to be the sites of ABA action on IAA transport.

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Abscisic Acid 對 Auxin 在綠豆下胚軸切節中運移的影響

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ABA 10^{-5} M 抑制 IAA-2-^{14}C 在綠豆 (*Vigna radicatae* L.) 下胚軸切節中之運移強度約51%，和運移密度 68%， 但對運移速度和總吸收量沒有影響。進一步探出 ABA 抑制 IAA 運移密度的原因發現：ABA 促進 IAA-1-^{14}C 的去羰基，抑制 IAA-2-^{14}C 與細胞膜結合。