IAA concentration and polar transport in cucumber fruit

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(Received December 25, 2022)

Summary

In cucumber fruit, the concentration of Apoplast (AP) solution was higher than that of Symplast (SY) solution at all three sites, both in the anthesis and in the pulp. In the pericarp, IAA uniformly migrated toward the stem at all three sites. On the other hand, IAA moved toward the floral side in the basal and apical portions of the fruiting body. The vegetative organ, the vine, showed the highest capacity for polar transport toward the root.

Bull.Facul.Agric.Niigata Univ., 75:1-5, 2023

Key words : Auxin, Apoplast, Symplast, Indole-acetic acid

Introduction

Indole-acetic acid (IAA) is synthesized in meristematic tissues and young tissues such as stem tips, juvenile buds, and immature seeds, and has a profound effect on stem elongation and organ differentiation (Wareing and Phillips, 1981). Only IAA, a plant hormone, is transported from the apex in a polar manner.

It has been reported that the receptor of IAA is located on the plasma membrane and its binding site is directed toward the outer apoplast (AP) (Venis et al., 1990). In pumpkin hypocotyls, the concentration of IAA in the AP, the outer plasma membrane fluid, is several times higher than that in the SY, the inner plasma membrane fluid, suggesting that the plant hormone in the AP is directly involved in plant development (Turusaki et al., 1997). Most of the studies on IAA have been conducted on sprouts, and there are no reports on fruits.

Therefore, in this experiment, cucumber fruits were used to compare the difference in concentration between AP and SY liquids in the fruit and to analyze the ability of polar transport.

Materials and Methods

Experiment A : Determination of IAA

Fruit material treatment method

House-grown cucumbers (rootstock, Super Yunryu; scion, Hikarisokusei) obtained from a cucumber farmer in Niigata City were used as fruit material. Twenty-four fruits, each approximately 20 cm long, were selected, packed in ice, and brought to the laboratory immediately.

Fruits were divided into three parts by length in the longitudinal direction (Fig, 1.). From the stem side, they were called 'base', 'middle' and 'apical'. The basal 2 cm and apical 2 cm of the fruit were discarded.

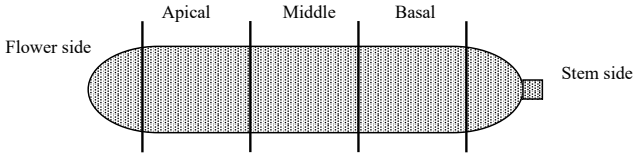

Fig. 1. Cucumber fruit part.

How to collect AP and SY solutions

AP and SY solutions were collected from pulp and placenta sections by centrifugation (Kojima et al., 2002). In a preliminary experiment, sections of various lengths were centrifuged at 5000 rpm (4528 × g) at 4° C for 5 minutes. When the length of the section exceeded one cm, the section could not withstand the centrifugal force, and the cells were broken and SY liquid was released. Therefore, the section length was set to one cm. The pulp and placenta were separated using a cork borer. Each section was arranged on a plastic mesh without gaps, and centrifuged at 4500 g for 5 minutes at 4° C to collect the AP solution.

After the AP solution was collected, the sample sections were quickly frozen in liquid nitrogen, and after thawing, they were again centrifuged under the same conditions to collect the SY solution. The collected solution was frozen and stored at -40° C until extraction.

Extract purification

After thawing the frozen SY solution, 200 pmol of stable isotope $^{13}C_6$-IAA (purity 99%, MSD isotopes; Montreal, Canada) was added as an internal standard, ascorbic acid as an antioxidant, and polyvinylpyrrolidone (PVP) as a phenol adsorbent was added. The sample solution was adjusted to pH 2.8 and suction filtered through a 0.22 μm membrane filter. Filtrates were extracted with diethyl ether and passed

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through a 0.22 μm syringe filter. The IAA fraction was collected by high-performance liquid chromatography (HPLC) equipped with an Inertsil OSD-3 column (5 μm, 6.0 mm i. d. ×250 mm; GL Sciences). For further purification, the IAA fraction was collected by HPLC equipped with an Inertsil Ph-3 column (4.6×250, GL Sciences). For the AP solution, purification by the Inertsil Ph-3 column in the second step was omitted.

LC-MS analysis

Liquid Chromatography-Mass Spectrometry: (LC-MS) (M-1200H; HITACHI) was used to quantify IAA (Kojima and Tamura 2001). Atmospheric Pressure Chemical Ionization (APCI) was used, the measurement mode was Selected ion monitoring (SIM) mode, and the polarity was set to Negative Mode (Kojima et al., 2003). A column [Cadenza (5 μm, 4.6 mm i. d.×250 mm; Imtakt)] was installed. The drift voltage he set to 30 V, which is optimal for IAA. IAA and ^{13}C-IAA molecular ions (IAA 174, ^{13}C-IAA 180) and characteristic fragment ions (IAA 144, ^{13}C-IAA 150) were monitored. The number of scans, the number of measurement points, [using (samples)], and the number of sweeps [with dwell count] were set to keep the time required for scanning each ion constant. The number of scans was set to 4, the number of measurement points to 40, and the number of sweeps to 7000.

Experiment B: IAA polar transport in cucumber fruit

A dilute solution of [2-^{14}C]IAA (Indole acetic acid [2-^{14}C]; American Radiolabeled Chemicals Inc) ([2-^{14}C]IAA: pure water = 2 μl: 3 ml diluted) was put into a styrene screw bottle (500 μl was placed in a 5 ml volume (upper body diameter×lower body diameter×height mm 24.2 × 23.6 × 38.0) (Kojima, et al., 2002). A cucumber fruit was divided into three equal parts along the length of the long axis, and designated as 'Basal', 'Middle', and 'Apical' (Fig, 2A.).

Using the central part of each part, it was cut into 2.5 cm long sections and a cork borer was used to separate the pericarp and the placenta (Fig, 2B.). Each was then cut in half. After measuring the fresh weight, the sections were immersed in a styrol bottle for 7 hours in the basal direction (toward the stem side) and the apical direction (toward the flower side) (Fig, 2C.). During immersion, IAA is easily oxidized and decomposed by light and oxygen in the air, so it was stored in an incubator without lighting (25° C).

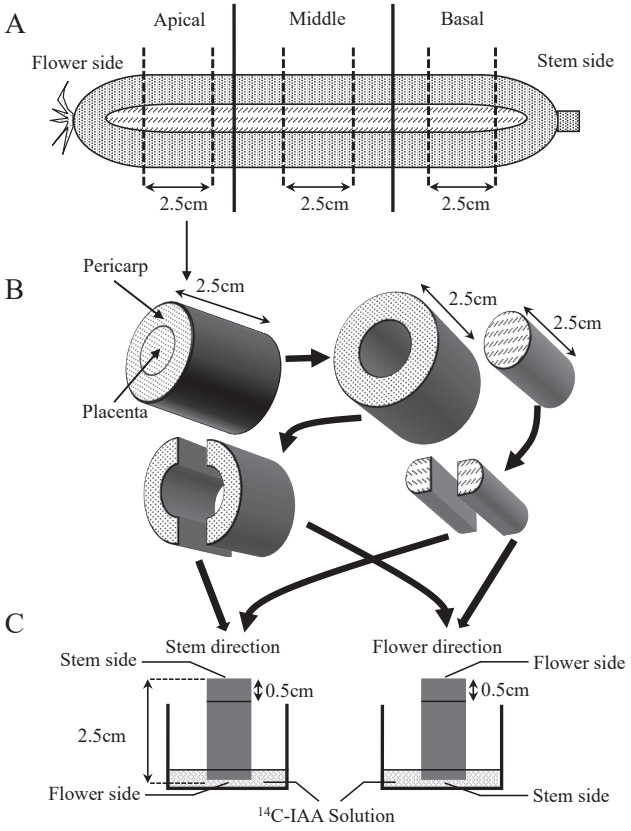

Fig. 2. Concentration of IAA in AP solution and SY solution in the placental part of cucumber fruit.

The solution of 24 fruits was mixed into one and analyzed. An internal standard was added, extracted with diethyl ether, fractionated, purified by HPLC, and quantified by LC-MS.

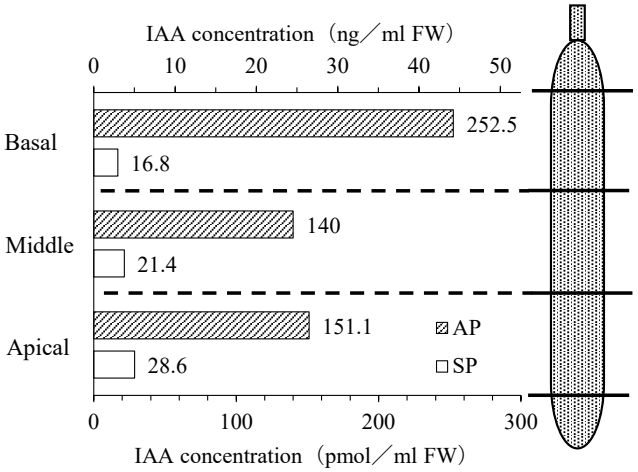

Fig. 3. IAA concentration of AP solution and SY solution in pulp of cucumber fruit.

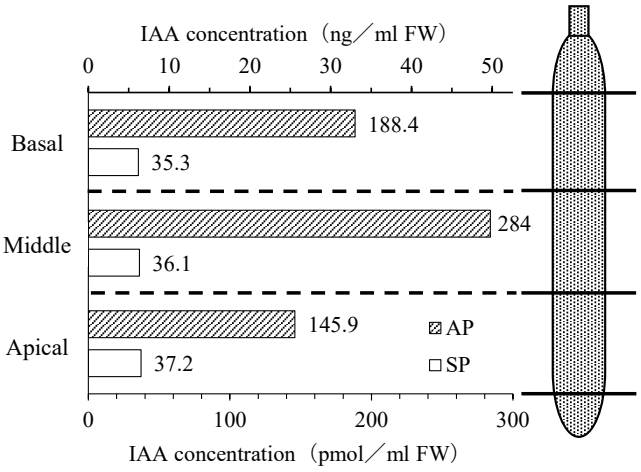

Fig. 4. Schematic diagram of experimental manipulation of IAA polar transport in cucumber fruit.
A: The fruit was divided into three equal parts along the length of the long axis, and designated as Basal, Middle, and Apical. A 2.5 cm long section was cut from the middle of each segment.
B: Each section was divided into pericarp and placenta using a cork borer, and then each section was cut in half lengthwise.
C: Each section was immersed in a styrol bottle for 7 hours in the basal (toward the stem) and apical (toward the flower) direction. After immersion, the sections were taken out and a 0.5 cm section was cut from the non-immersed side.

After immersion, a 0.5 cm section was cut off from the non-immersed side and placed in a liquid thin counting vial containing 5 ml of ACSII (Aqueous Counting Scintillant; Amersham) (same diameter x total height mm, 28 x 61) was immersed for 4 days and stirred daily so that the amount of radioactivity did not change.

The amount of radioactivity eluted was measured over time, and after the elution was stabilized, all vials were measured for radioactivity (dpm; Disintegration/minute disintegration rate) with a liquid scintillation counter (LSC-6000; Aloka Co., Ltd.).

The BG value was subtracted from the measured total radioactivity and divided by the cross-sectional area and time values. The calculated value (dpm/cm2/h) was applied to the dpm-pmol conversion formula (pmol = 0.011 dpm) to convert the unit. The IAA polar transport capacity of each site was calculated from the difference in radioactivity between the two sections.

Results and Discussion

Experiment A: Quantification of endogenous IAA in cucumber fruit

The IAA concentration was higher in the AP solution than in the SY solution at all three sites in the placenta (Fig, 3.). It was 15-fold at the base, 6.5-fold at the middle, and 5.3-fold at the apical.

Concentrations of IAA in the pulp were also higher in all of the three sites, and AP solution was higher than SY solution (Fig, 4.). 5.4 times at the base, 7.9 times at the middle and 3.9 times at the apical.

The endogenous IAA concentration in each part of the cucumber fruit was 4-15 times higher in the AP solution than in the SY solution, similar to the IAA concentration in the hypocotyl of pumpkin (Turusaki et al., 1997). From this fact, the future physiology of IAA, measurement of the IAA concentration in the AP solution is important for elucidating its action. In SY, IAA concentrations in the placenta and pericarp were similar. This suggests that in parthenocarpic cucumber fruits, IAA is synthesized in the whole fruit rather than in specific parts of the fruit.

Experiment B Ability of IAA polar transport in cucumber fruit

From the weight of each section, the cross-sectional area was calculated with the specific gravity set to 1. Table.1. shows the cross-sectional area of each section and the amount of transported radioactivity derived from the radioactivity value (dpm).

In the pericarp part, in three parts. IAA uniformly migrated toward the stem side. On the other hand, in the placenta, IAA moved toward the flower at the middle and apical. In the vine, which is a vegetative organ, the ability of polar transport toward the root was the highest.

Results were mixed in the placenta of cucumber fruit, suggesting that there was almost no polar transport of IAA in the placenta and the majority was diffusion. On the other hand, in the pericarp part, the polarity was seen toward the stem side, albeit moderately. There are no reports of polar

Table 1　Levels of ^{14}C-IAA transport in fruits of cucumber

Parts	Section	Direction	Levels of Transport (pmol/h/cm^2)	Differences of transport levels[c] (pmol/h/cm^2)
Basal	Pericarp	Flower[a]	4.3 ± 1.0	12.4 ± 3.6[d]
		Stem[b]	16.7 ± 4.3	
	Placenta	Flower	19.4 ± 11.5	2.7 ± 17.5
		Stem	22.1 ± 6.6	
Middle	Pericarp	Flower	10.4 ± 5.1	9.2 ± 3.3
		Stem	19.6 ± 6.0	
	Placenta	Flower	17.9 ± 7.2	-8.5 ± 6.2[e]
		Stem	9.4 ± 2.1	
Apical	Pericarp	Flower	20.2 ± 4.1	17.4 ± 21.5
		Stem	37.6 ± 20.5	
	Placenta	Flower	20.3 ± 8.1	-5.5 ± 5.4
		Stem	14.8 ± 6.7	
Vine		Top	16.1 ± 8.4	91.4 ± 26.6
		Root	107.6 ± 20.2	

[a]Measurement of transport toward flower side (stem side immersed in ^{14}C-IAA diluted solution)
[b]Measurement of transport in the direction of the stem (flower side immersed in ^{14}C-IAA diluted solution)
[c]Difference in amount of transported IAA: polar transport capacity
[d]A positive value (eg, 12.4 ± 3.6) indicates that polar IAA transport is toward the stem side.
[e]Negative values (eg, -8.5 ± 6.2): indicate polar IAA transport toward the flower side.
The mean ± SE of four intercepts is shown.

transport in fruit (Naylor, 1984).

Cucumbers grown in greenhouses at this time are almost parthenocarpic because there are no insects that promote pollination. Therefore, in fruits, plant hormones are usually synthesized in seeds, but in this material, seeds do not exist. Therefore, the following can be considered: 1) IAA synthesized in each part of the fruit slowly diffuses physically in the placental part. 2) IAA that has moved to the pericarp part gradually moves toward the vine (fruit stem) by polar transport. 3) In the vine, IAA flowing from the pericarp is transported toward the root by strong polar transport in the thin vine.

Acknowledgments

The Author thanks Ms. K. Nagahama for preparing the graphs.

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キュウリの果実内の IAA 濃度と極性移動 .

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（2022年12月25日受付）

要　約

キュウリ果実において、濃度は胎座と果肉においても３つの全ての部位で、アポプラスト（AP）液はシンプラスト（SY）液よりも高い濃度であった。果皮部分において、３つの部位で。IAA は一様に茎側に向かって移動していた。一方、胎座部において、基部と先端部では花側に向かって IAA は移動していた。栄養器官であるつるでは根方向への極性輸送の能力が最も高い値であった。

キュウリ果実で定量を行った主要な植物ホルモンにおいて、果肉部と胎座部とも、AP 溶液の方が SY 溶液よりも高い濃度であった。

新大農研報、*75:1-5*、*2023*

キーワード：オーキシン、アポプラスト、シンプラスト、インドール

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