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INTRACELLULAR LOCALIZATION OF NATIVE AUXIN IN *AVENA* COLEOPTILE

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Intracellular localization of the native auxin in the *Avena* coleoptile tip was investigated by separating cellular fractions by differential centrifugation. Each fraction was extracted with ether and the auxin activity was measured by the sensitized *Avena* curvature test. After the removal of the native free auxin, each fraction was alkaline-hydrolyzed, and from these hydrolyzates the bound auxin was extracted with ether and its activity was measured. Both the native free auxin and the native bound auxin in these extracts were identified as IAA by paper chromatography. The results show that the native free auxin occurs only in the supernatant soluble cytoplasm, and that the native bound auxin localizes also in the supernatant. The distribution of the externally applied IAA was also investigated.

It has been reported on the basis of chromatographic evidence that IAA occurs in more than twenty plant species. Although various aspects of physiology of IAA have been reported (*1*), nothing has been known about the reaction which is primarily brought about by IAA when IAA causes physiological changes in a plant. With the hope of contributing to the elucidation of this problem, the present study was performed to make clear the localization of native auxin in different subcellular bodies which were separated by differential centrifugation using *Avena* coleoptiles as material.

MATERIAL AND METHODS

Fractionation

The coleoptiles of *Avena sativa* (Victory No. 1) were used throughout this study. *Avena* seedlings were grown at 27° in darkness, and when

Abbreviations: IAA, 3-indoleacetic acid; 2, 4-D, 2, 4-dichlorophenoxyacetic acid.

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the height of the seedlings reached 2–3 *cm*, the tips of coleoptiles, 1 *cm* in length, were harvested and the primary leaves were pulled out from the coleoptiles. For separation of the intracellular organelles, tissues were ground in a chilled blendor for several minutes with an equal volume of cold KH_2PO_4-Na_2HPO_4 buffer, 0.03 *M*, pH 5.5, containing 0.2 *M* sucrose. The brei was filtrated through 200 mesh muslin and the filtrate was fractionated by differential centrifugation as indicated in Table II.

Auxin extraction

1. Extraction of native free auxin—Each of the five fractions obtained by differential centrifugation was extracted three times with peroxide-free ether in a separatory funnel. The three ethereal extracts were combined and extracted three times with a small volume of 8% sodium bicarbonate. The three aqueous extracts were combined together, brought to pH 6.0 with 15% tartaric acid solution, and extracted three times with ether. The ether extracts were combined and evaporated to dryness.

2. Extraction of native bound auxin—The extraction of native bound auxin was carried out according to a modified method of YAMAKI and NAKAMURA (*2*). After the removal of native free auxin as described above, each fraction (A to E of Table II) was mixed with an equal volume of 3/20 *M* borax-sodium hydroxide buffer solution, adjusted to pH 9.5 and hydrolyzed for 5 minutes at 120°. Each hydrolyzed fraction was adjusted to pH 6.0 by the addition of 15% tartaric acid, and extracted with ether three times. The three ether extracts of each fraction were combined and evaporated up to dryness.

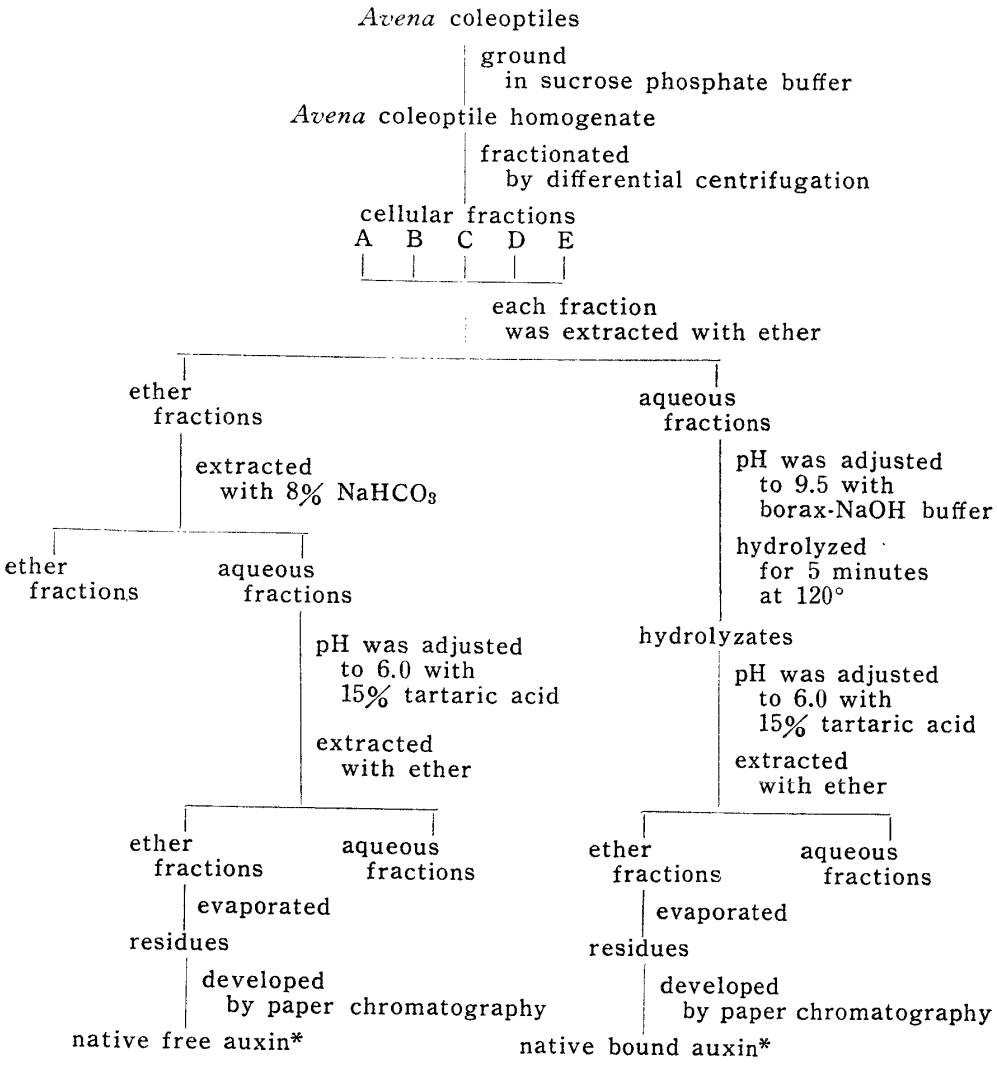
Assay of auxin activity

The technique of paper chromatography was used for the identification and purification of the extracts. The residual substances obtained by ether evaporation were used for the analysis. Chromatographical analysis was carried out by the method of SHIBAOKA and YAMAKI (*3*). Toyo No. 51 filter paper strips, 3 *cm* in width, were used and the ascending method was applied. As developing solvent system, *iso*-propanol-water-ammonia (10 : 1 : 1 v/v) was used. The solvent was allowed to run 19.5 *cm* up from the start line and the strips were dried. The chromatogram, having 20 *cm* in length from 5 *mm* below the start line up to the solvent front, was cut crosswise into 10 equal pieces and each division was wetted with 0.2 *ml* of water. Fifteen blocks of 1.5% agar ($2\times2\times2$ mm^3) were placed on each of the 10 pieces of the wetted filter paper for 3 hours. To the 15 agar blocks removed from the filter paper, a small drop of 5×10^{-3} *M* $FeSO_4$ solution was added and these agar blocks were used for the sensitized *Avena* curvature test (*3*). The pure IAA spot

was determined colorimetrically with SALKOWSKI-TANG's reagent. These methods are summarized in Table I.

TABLE I

Extraction of native auxin



* Auxin activity was tested by sensitized *Avena* curvature test.

RESULTS

Identification of native auxin

Both free and bound auxins extracted from *Avena* coleoptiles were identified as IAA by the chromatographical analysis. In both cases,

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the auxin activity was found on zones No. 5 and No. 6 of the paper chromatograms as indicated in Fig. 1. The R_f values of these were, however, lower than that of pure IAA which was developed with the

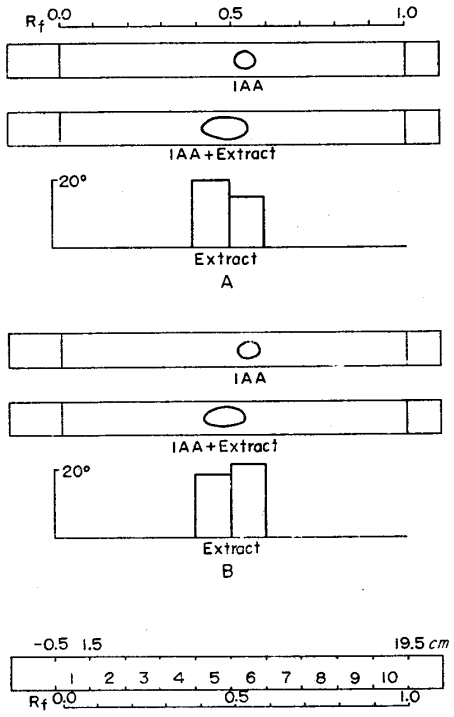

Fig. 1. The chromatograms of the extracts from *Avena* coleoptile tips. The solvent system was *iso*-propanol-water-ammonia (10: 1: 1 v/v). Auxin activity was measured by the sensitized *Avena* curvature test and expressed as the degree of the coleoptile curvature. The paper strip presented at the bottom indicates the distance (in *cm*) of each numbered zone from the starting line. A: The activity of the native free auxin; the fresh weight was 22 *g*. B: The activity of the native bound auxin; the fresh weight was 77 *g*.

same solvent system. These differences in R_f values suggest that the contaminations in the extracts might have brought about the lowering of R_f values of auxin on the strips. To solve this question, the following treatment was made. A mixture of IAA and the extract was put on the starting line and developed with the same solvent system in each case. The result presented in Fig. 1 shows that contaminations in each extracts induced the lowering of R_f value of IAA as suggested above, and it is concluded that the R_f value of the native free auxin as well as of the native bound auxin in the extract coincides with that of IAA used as control. For both auxins, this experiment was repeated three times and the same results were obtained. Accordingly, both free and bound auxins were identified as IAA.

The following experiment was carried out as the next step, because it was feared that the contaminations in impure extracts might inhibit the auxin activity in the curvature test. The auxin activities of four series of agar blocks, *i. e.*, agar blocks placed on: zone No. 5 + 6 of the developed chromatogram wetted with a small amount of water, the same zone wetted with the same amount of 0.05 *ppm* IAA, a piece of filter paper wetted with water, and a piece of filter paper wetted with 0.05 *ppm*

IAA, were compared by the sensitized *Avena* curvature test. The result showed that the IAA activity was not inhibited by the contamination in the extracts.

Distribution of native free auxin and native bound auxin

The distribution of the native free and bound auxins are given in Table II. Because it is obscure whether the free auxin still remains in

TABLE II

Distribution of native auxin extracted from Avena *coleoptile tips, ca. 80 g in fresh weight*

Fraction	Centrifugation	Description	Auxin activity (*Avena* curvature in degree)	
			Free auxin	Bound auxin
A	10 *min* 1,000×*g*	Plastids, nuclei, wall fragments	0.0°	0.0°
B	10 *min* 4,500×*g*	Plastids	0.0	?[a]
C	30 *min* 14,000×*g*	Mitochondria, plastids	0.0	0.0
D	30 *min* 100,000×*g*	Microsomes	0.0	0.0
E	No. 5[b] Supernatant	Soluble cytoplasm, cell sap	14.1	17.7
	No. 6[b]		0.0	15.7
0.05 *ppm* IAA			18.6°	
Water			0.0	

[a] Low auxin activities were observed in two out of eight experiments.
[b] The zone number of the paper chromatogram.

the aqueous fraction after its removal by ether extraction, the following experiment was performed. After the free auxin was extracted three times with ether by a separatory funnel, the fourth extraction was carried out and the auxin activity in the fourth ether extract was tested. But no auxin activity was observed. The result shown in Table II indicates that the native free auxin localizes only in the supernatant soluble cytoplasm and that the native bound auxin occurs also in the supernatant fraction. Little auxin activity was observed in the other fractions. The datum reported here is an example of eight experiments which all gave similar results. The quantitative ratio of the free auxin to the bound auxin was not determined in the present experiment.

Distribution of externally applied auxin

The distribution of externally applied IAA was examined. The preliminary experiments showed that the optimum IAA concentration was

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3–5 *ppm* for the elongation of coleoptiles, and that the coleoptiles which reached 2–3 *cm* were more elongated with IAA than the shorter coleoptiles. Accordingly, in this experiment 1 *cm* tips from 2–3 *cm* coleoptiles, grown under the condition described above, were floated on 5 *ppm* IAA solution containing 2% sucrose for 3 hours in darkness at 27°. The control tips were floated on 2% sucrose solution. At the end of the incubation period, the tips were thoroughly washed with distilled water and the fresh weight and the length were measured. Then the auxin was extracted after the fractionation and its activity was measured in the same way as used for the native auxin. The result is shown in Table III. In the supernatant soluble cytoplasm of the treatment, the

TABLE III

Distribution of externally applied IAA

Fraction		Auxin activity (*Avena* curvature in degree)			
		Free auxin		Bound auxin	
		Treatment[a]	Control[b]	Treatment[a]	Control[b]
A		0.0°	0.0°	0.0°	0.0°
B		0.0	0.0	0.0	0.0
C		0.0	0.0	0.0	0.0
D		0.0	0.0	0.0	0.0
E	No. 5[c]	17.2 (0.37°/*g* FW)	4.7 (0.14)	6.6 (0.14)	5.6 (0.17)
	No. 6[c]	15.0 (0.32)	8.3 (0.25)	6.0 (0.13)	5.7 (0.17)
0.05 *ppm* IAA			17.8°		
Water			0.0		

[a] The tips of material were floated on 5 *ppm* IAA solution containing 2% sucrose for 3 hours in darkness at 27°.
The fresh weight was 46 *g*.

[b] The tips of material were floated on sucrose solution.
The fresh weight was 33 *g*.

[c] The zone number of the paper chromatogram.

free auxin activity was roughly twice that of the control (Table III). In the case of the bound auxin, little difference between the treatment and the control was found. The distribution of the externally applied IAA cannot be discussed from the results of the present experiment.

DISCUSSION

It may be concluded that in the *Avena* coleoptile cells the native free auxin as well as the native bound auxin is IAA, and that both

auxins occur in non-particulate soluble cytoplasm and/or cell sap.

Little investigations have been made on the intracellular localization of native auxin in higher plants. GALSTON *et al.* (*4*, *5*) demonstrated that labeled 2, 4-D attached itself to the soluble cytoplasm and changed the nature of protein in the cytoplasm of pea stem cells, and it was reported by THIMANN and BETH (*6*) that nucleus is not the primary site when IAA reacts as hormone in *Acetabularia*.

If the primary act of the externally applied IAA is the formation of the bound auxin, which is probable but not definitely proved in the present study, the next step of our study which seems to be relevant may be that regarding the elucidation of the relationship between the bound auxin and IAA. In the present experiment, the materials examined were mixtures of the meristematic region of the coleoptile tip and the elongation region of the coleoptile. If the physiologically different regions are divided and studied separately, the difference in the physiological roles of the free and bound auxins in the *Avena* coleoptile will be made more distinct.

The study reported in the present paper was conducted as a part of development of the following works: that of YAMAKI and NAKAMURA (*2*) who reported that a fairly large amount of ether extractable free auxin was found in maize embryo, and that, although in a small amount (20% of that of free auxin), bound auxin was obtained by alkaline hydrolysis from the embryo of maize, that of HOTTA (*7*) who demonstrated that the ether extractable auxin plays some role in the differentiation and the growth of the gametophyte in a late stage of development, and that the bound auxin has close relations to the change from the one-dimensional growth to the two-dimensional growth in *Dryopteris erythrosora*.

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