TRANSPORT, BINDING, AND DECARBOXYLATION OF CARBOXYL-LABELED IAA-^{14}C IN INTACT PEA ROOTS

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SUMMARY

Carboxyl-labeled IAA-^{14}C was predominantly transported in the acropetal direction, when applied to intact vertical or horizontal pea roots. The velocity was several cm per hour, suggesting transport in the phloem. A large proportion of the auxin entering the roots became bound. The major part of the IAA-^{14}C applied to the root surface was decarboxylated. Three methods of auxin application were used, viz. in 1 µl droplets of an aqueous solution, in lanolin paste, and by means of anion-exchange resin beads.

1. INTRODUCTION

The study of the transport of labeled indoleacetic acid (IAA-^{14}C) in roots has been restricted to segments. It has been shown that this auxin moved predominantly in the acropetal direction through these segments, cut either from the elongation zone of the roots (PILET 1964, *Lens culinaris;* KIRK & JACOBS 1968, *Lens culinaris* and *Phaseolus vulgaris*; WILKINS & SCOTT 1968, *Zea mays*, *Avena sativa*, *Triticum vulgare* and *Helianthus annuus*; SCOTT & WILKINS 1968, *Zea mays*; IVERSEN & AASHEIM 1970 and AASHEIM & IVERSEN 1971, *Helianthus annuus* and *Brassica oleracea*), or also from older parts of the roots (BONNETT & TORREY 1965, *Convolvulus arvensis;* CANE & WILKINS 1970, *Zea mays*). In other experiments no clear polarity of IAA transport was found in segments cut from the elongation zone (YEOMANS & AUDUS 1964, *Vicia faba*; BONNETT & TORREY 1965, *Convolvulus arvensis*; WILKINS & SCOTT 1968, *Pisum sativum*). If, however, as HILLMAN & PHILLIPS (1970) demonstrated with pea roots segments, the IAA concentration was higher and the transport period longer than usual, these segments also transported more IAA acropetally than basipetally.

Suggestions on the transport of indoleacetic acid applied to *intact* roots, although again restricted to the elongation zone, were presented by CHOLODNY (1931) and by NAGAO & OHWAKI (1968), who assumed that the basipetal transport was better than the acropetal transport in this region.

No data are available at present on the transport of IAA, when applied to intact roots, along some length of these roots in the two directions. The experiments described below were therefore aimed at determining the distribution of IAA-^{14}C, following the application, halfway apex and base, in intact pea seedling roots. Decarboxylation of IAA is known to occur when added to tissue homogenates or cut surfaces of tissue segments. In this connection it was decided

to determine whether the decarboxylation could also be accomplished by intact roots.

2. MATERIALS AND METHODS

Two-day-old roots of *Pisum sativum* cultivar 'Vlijmse Gele Krombek' of about 45 mm length were used. The seeds were soaked in aerated tap water for 20 hours, then placed in moist sand over vertical holes and allowed to germinate in the dark until the roots had the desired length. All handling occurred at 24 °C and 85 per cent relative humidity under red light (Philips TL 40 watt, colour 15, filtered through 3 mm thick plexiglass Röhm and Haas nr 501). During the transport period the roots were in the dark. The seedlings were pinned on frames with their roots either vertical or horizontal and placed in 700 ml vessels, closed with a glass-plate and silicone grease, in air with saturated humidity. A series of 10 roots was placed in each vessel. Routinely three series of 10 roots received the same treatment. Each of the glass-plates contained a porthole covered with a silicone rubber septum.

The IAA-^{14}C, from The Radiochemical Centre, Amersham, England, had a specific activity of 57 mCi/mM and a checked purity of almost 99 per cent. The sample was stored in acetonitrile at –20 °C. For the experiments dilutions were made in distilled water at 60 °C where the acetonitrile was allowed to evaporate. Three methods of auxin application were used. First, a 1 µl droplet of an IAA-^{14}C solution in distilled water was applied to the root surface between two previously applied rings of lanolin, 2 mm apart. The droplets contained 4400 dpm on the average. Second, equal weights of lanolin and the IAA-^{14}C solution were mixed. To each root a narrow ring of the paste of approximately 2 mg was applied, also containing about 4400 dpm. Third, anion-exchange resin beads (Dowex I–X8) were put in an IAA-^{14}C solution for 16 hours. At that time 95 per cent of the radioactivity of the IAA-^{14}C solution was attached to the beads and no further uptake occurred. Of the remaining radioactivity 4.2 per cent stayed in the solution and 0.8 per cent became spontaneously decarboxylated and was measured as $^{14}CO_2$. Before the experiments the beads were rinsed in distilled water and next applied to the roots; two beads to each root, opposite each other. The beads easily adhered to the root surface. The locus was marked with a tiny dot of carbon powder in paraffin oil.

After the experimental period, the ^{14}C still attached to the beads was determined in a dioxane-based scintillation liquid (800 ml of dioxane, 160 ml of cellosolve, 48 g of naphthalene and 5 g of Premix-M), to which the thixotropic gel Cab-o-sil was added in order to prevent sedimentation of the beads. It was observed that the number of counts obtained from the beads increased with increasing time in the scintillation liquid until, after a few weeks, the counts did not further increase and, in the case of control beads, corresponded within 5 per cent with the amount originally taken up by the beads. Consequently, at this time the activity of the beads used in the experiments was accepted to represent the amount of IAA-^{14}C still attached to them after the exchange period at the root surface.

In all cases the IAA-^{14}C was applied at 20–22 mm from the apex. At the end of the experiments the roots had elongated a few mm and the original site of application was at 22–24 mm from the apex. After the transport period, the roots were cut into a number of segments. The segments were first collected in acetonitrile at –40 °C. They were next extracted in the acetonitrile at 65 °C during 1.5 hours. The acetonitrile extract was decanted in a counting vial with the dioxane-based scintillation liquid. The tissue was washed with fresh acetonitrile and the washings were also added to the same vial. To obtain the more firmly bound radioactivity the tissue was ground in 2N NaOH and left at room temperature for 48 hours. In part of the experiments the tissue was put in the solubilizer soluene (Packard) and kept for 20–40 hours at 60 °C to solubilize the tissues. All extraction tubes were washed twice with 1 ml of methanol. The washings and the extracts were put in the scintillation liquid as mentioned above. In the case of the NaOH extracts Cab-o-sil had to be added. The yield of ^{14}C was almost equal in the two extracts.

The decarboxylation of the IAA-^{14}C was determined as follows. The gas in the vessels was stirred by means of a 10 ml syringe and a long needle, before the frame with the roots was removed. Then 3 samples of 10 ml each were taken from the vessel and injected into a jar with 10 ml of a 30 per cent KOH solution at the bottom. This jar was left at room temperature for two days. Then the $^{14}CO_2$ trapped in the KOH was determined by adding samples to vials with the dioxane-based scintillation liquid with Cab-o-sil.

All samples were counted for 10 minutes. Occasionally they were counted for a second time. The counts were corrected for background and, with the aid of an AES ratio/efficiency curve, for quenching.

For thin-layer (Kieselgel G) chromatography the acetonitrile extracts were put directly on the plates. The NaOH extracts were acidified to pH 3 with 1N H_2SO_4 and extracted 3 times with 3 ml of ether, the ether fractions were collected and the ether was evaporated to a small volume and applied to the plates. The solvent was isopropanol/ammonia/water (8:1:1). Autoradiographs of the plates were made on X-ray film (Kodak) for 2 to 4 weeks.

3. RESULTS

3.1. The distribution of IAA-^{14}C in intact pea roots following its application between apex and base

The distribution of the radioactivity in vertical and horizontal roots two hours after the IAA-^{14}C has been applied at 20 mm from the apex is illustrated in the *figs. 1a, 1b,* and *1c.* Clearly, the auxin has been translocated both in the acropetal and in the basipetal direction and has reached the apex and the epicotyl. It is also clear that more ^{14}C moved to the tip than to the base. The auxin distribution showed a steep gradient from the site of application in the two directions, but at about 10 mm from this site the distribution was approximately linear along some length of the root. Accumulation of the acropetally transported IAA-^{14}C occurred in the tip, particularly in the 0–2 mm apical region where

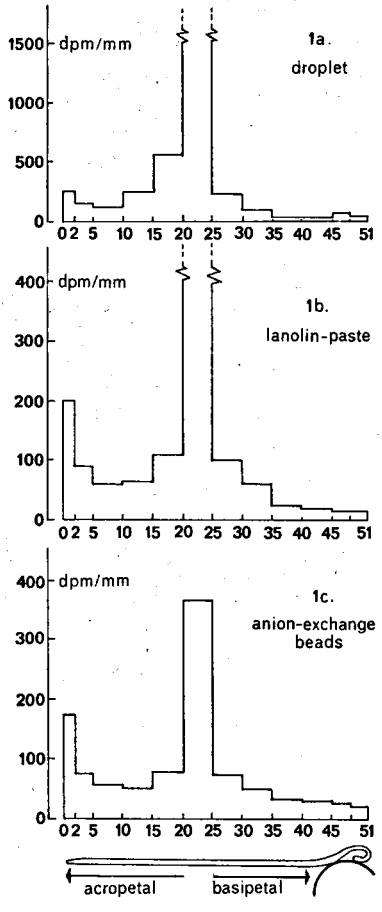

Fig. 1. The distribution of radioactivity in the different segments cut from intact, vertical and horizontal pea roots, after a 2-hour transport period. The ^{14}C was first extracted by acetonitrile and followed by 2N NaOH or soluene extraction. The two activities were summarized. The IAA-^{14}C was applied at 20–22 mm from the apex in a droplet of an aqueous solution, in lanolin paste or by means of anion-exchange resin beads. The 45–48 mm segment comprised the collar and the 48–51 mm part was the epicotyl. The count rates are expressed as dpm per 30 segments. Note the different scale of fig. 1a.

most of the cells of the meristem are located. No such accumulation of ^{14}C was found in the epicotyl. The polarity of the auxin distribution as illustrated in the figures is evident.

Comparison of the *figs. 1a* and *1b* shows that in both cases relatively large amounts of radioactivity were present in the region of application after 2 hours (far off-scale peaks), although the amount introduced by the paste was much less than by the droplet (the figure does not show this; see *table 1*). In the other regions, except in the basal regions where the differences were small, the radioactivity was much higher when a droplet was applied than when the same amount of ^{14}C was given in lanolin paste (note different scale of *fig. 1a*). The auxin applied in lanolin paste entered gradually, because it was not all at once in contact with the root surface. Remarkably, the anion-exchange beads loaded with IAA-^{14}C (35000 dpm per bead) did not cause such a large accumulation of radioactivity at the site of application. *Fig. 1c* shows only a relatively small

on-scale peak. The amounts of ^{14}C in the other segments were as large as when the auxin was applied in lanolin paste, where much more auxin entered the roots. In other words, a relatively large part of the IAA-^{14}C absorbed by the roots was transported. Of the total radioactivity found in the roots approximately 53 per cent was present in the regions other than the region of application (20–25 mm); this was roughly estimated 12 per cent when the auxin was applied in paste and 15 per cent in the case of the droplets.

It is noteworthy that apparently the IAA-^{14}C of the beads was exchanged at the root surface. The restricted amount of auxin that entered the roots could have been the result of the small contact area between the beads and the root surface and possibly also by the availability of exchangeable molecules in the root exudate.

Fig. 2 demonstrates how the IAA-^{14}C (applied in a droplet) increased in the different segments, at least during the first few hours. The amounts moving acropetally were very much larger than those moving basipetally, where the increase with time was only very slight. Thus, again the polar transport was very clear. The figure shows also how the accumulation in the tip developed.

After 30 min already some radioactivity was found in the extreme tip (the 0–1 mm segment in fact). So the velocity of the auxin transport was at least several cm per hour. Acetonitrile and NaOH extracts were made from the roots of 2-hour experiments where the IAA-^{14}C was applied in a droplet. The region of application (20–25 mm) was discarded. The chromatograms of these extracts showed only one zone of radioactivity at Rf 0.45 to 0.52, coinciding with the Rf of fresh IAA-^{14}C. Therefore no metabolites could be detected at this time.

3.2. The binding of IAA-^{14}C in different regions of intact pea roots

Only part of the IAA-^{14}C in the different root segments could be extracted by acetonitrile, or by ethanol (48 hrs at 4 °C). This portion of the IAA will be referred to as free IAA, while the remainder will be called bound IAA. In *table 1* are summarized the data of the radioactivity extracted by acetonitrile and 2N NaOH or soluene respectively.

When the IAA-^{14}C was applied in a droplet, i.e. of the three methods the

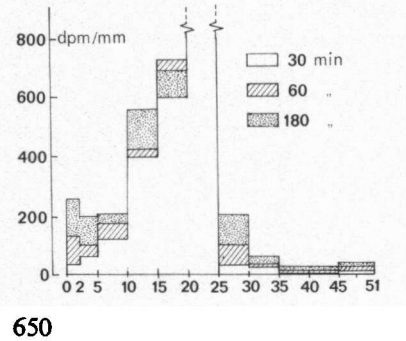

Fig. 2. The distribution of the radioactivity in intact vertical pea roots at 30, 60, and 180 min after auxin application at 20–22 mm from the apex. The auxin was applied in a droplet of an aqueous solution. The ^{14}C was extracted by soluene. Count rates are dpm per 30 root segments.

Table 1. Free (extracted by acetonitrile) and bound (extracted by 2N NaOH or soluene) IAA-^{14}C in the different segments cut from intact pea roots after a 2-hours transport period. The auxin was applied in a droplet of an aqueous solution, in lanolin paste or by means of anion-exchange resin beads. The count rates are expressed as dpm per 30 segments.

segments mm	droplet		lanolin paste		beads	
	free	bound	free	bound	free	bound
0–2	65	420	39	355	88	263
2–5	236	282	62	208	66	118
5–10	331	353	77	249	128	155
10–15	685	558	117	234	100	157
15–25+	20350	11500	–++	6340	1140	836
25–30	412	540	90	410	104	185
30–35	208	192	81	223	110	158
35–45	67	140	25	145	114	182
collar + epicotyl	65	112	16	198	37	93

+ The 15–25 mm segment included the site of IAA-^{14}C application.
++ The acetonitrile-extract of the tissue was mixed up with the extract of the lanolin paste.

greatest amount, the bound auxin exceeded the free auxin in the apical 2 mm and in the basal regions. At the site of application (the 15–25 mm segment) the free auxin exceeded the bound auxin. In the other segments the amounts of free and bound auxin were about equal. These results could be visualized as follows. In the region of application the supply of IAA strongly overloaded the binding capacity. In the tip, which included the meristem, the binding capacity was so great that the major part of the auxin arriving there was shifted into the bound state. At the basal end the relatively large part of bound IAA might be related to the low transport density in this region (cf. lanolin paste and beads below). The regions in between probably had intermediate conditions of auxin supply and binding capacity, so that about 50 per cent became trapped in the bound state. The IAA-^{14}C applied in lanolin paste or by means of beads, became bound for the major part in all segments. This was particularly clear when the auxin was applied in the paste. Again, the ratio bound/free was most extreme at the two ends. With the two latter methods smaller amounts of IAA-^{14}C were introduced into the roots than with the droplet method. The density of the auxin transport was less in these cases. This lower transport density coincided with a shift to more bound auxin.

3.3. The decarboxylation of IAA-^{14}C applied to the surface of intact roots

Decarboxylation of IAA is known to occur in tissue homogenates and at cut surfaces. The decarboxylation of the IAA-^{14}C, applied in the three different manners to the surface of intact roots, has been detected now and it appeared to be a very active process. *Table 2* presents the data of the $^{14}CO_2$ production. In all three cases substantial quantities of $^{14}CO_2$ were produced. The largest amount

Table 2. The distribution of the activity between root tissue, carbon dioxide, and the auxin source after a 2 hrs transport period. The original activity was approx. 132000 dpm in the droplet and paste method and about 1μCi at the beads. The count rates are expressed as mean dpm per 30 roots.

	tissue	$^{14}CO_2$	beads or paste	% recovery
droplet	41400	77240	–	90
lanolin paste	6700[1]	38140	91500[2]	100
beads	3730	43540	1837900	90[3]

[1] Bound IAA-^{14}C only.
[2] Acetonitrile extract of the lanolin paste and the tissue of the region of auxin application.
[3] Maximum recovery was less than 1 μCi; 5 per cent of the activity was either not taken up by the beads or spontaneously decarboxylated.

was produced when the IAA-^{14}C was applied in a droplet where, of the three methods, the amount of auxin applied to the roots at once, was largest. The $^{14}CO_2$ production was less in the two other cases where smaller quantities of auxin were applied. The portion of the IAA-^{14}C that became decarboxylated relative to the part that was found in the root segments was extremely large in the case of the beads method. In this case the supply to the root was the least abrupt, because the IAA-^{14}C first had to be exchanged against some other anion. The high rate of decarboxylation found suggested that an IAA-^{14}C molecule leaving the beads had a greater chance to be decarboxylated than an auxin molecule had in each of the other methods.

Apparently, the roots possessed a very effective means to prevent most of the IAA-^{14}C, when applied from the outside, to enter the root unaltered.

4. DISCUSSION

The results show that IAA-^{14}C applied to intact pea seedling roots was translocated both in the acropetal and in the basipetal direction in these roots, but also that the acropetal stream was much stronger than the basipetal one.

The transport velocity was, as *fig. 2* indicates, at least several cm per hour, which was clearly higher than the velocities of a few mm per hour usually found in root segments. The transport in root segments is known to occur in the parenchyma cells, but it has been demonstrated by ESCHRICH (1968) that IAA-^{14}C applied to intact *Vicia faba* plants moved in the phloem in both the acropetal and the basipetal direction. Transport velocities in the phloem are much higher than in parenchyma cells. LITTLE & BLACKMAN (1963) measured a velocity of 20 to 24 cm per hour in intact plants of *Phaseolus vulgaris*. On the basis of these facts we may assume that the transport of the IAA-^{14}C in the intact pea roots also occurred in the phloem.

The three different methods of IAA-^{14}C application were used, because some difference between them could be expected in their respective rate of auxin de-

liverance to the roots. The application of IAA by means of Dowex-I-X8 anion-exchange resin beads was introduced by GEE & GREYSON (1969). They put the spheres, loaded with IAA, on the longitudinally cut surfaces of split pea stalks. The response of the stalks was clear so that obviously exchange of IAA had occurred. We expected that exchange might also occur at the surface of intact roots, because roots exude numerous compounds. As the results demonstrated, IAA was exchanged and entered the roots. Compared with the two other methods a relatively large portion of the IAA-^{14}C absorbed by the roots was translocated, since no strong accumulation was found at the site of entrance.

This manner of auxin application thus disturbed the physiological situation least. The two other methods of auxin application, however, did not overload the transport system either since the amounts of ^{14}C extracted from the different segments were about equal for lanolin paste and beads and only about twice as much when the IAA-^{14}C was applied in a droplet. Most of the auxin entering the roots remained in a narrow region below the site of application, as is also known to happen to auxin applied to cut surfaces of tissue segments.

As seen in *table 1*, the major part of the transported IAA-^{14}C became bound, possibly to protein as was found to happen in *Avena* coleoptiles (WINTER & THIMANN 1966). Such binding was also indicated by the extremely high binding in the protein-rich cells of the 0–2 mm segments. The overall binding percentage in the roots was much higher than the 15 per cent found in the coleoptiles. The binding probably occurred in the parenchyma cells and not in the sieve tubes, since Eschrich reported that the bound auxin in *Coleus* stems was not found in these tubes.

The $^{14}CO_2$ data revealed that a large proportion of the IAA-^{14}C, when applied to intact roots, was decarboxylated. The chance of a molecule of IAA-^{14}C to become decarboxylated was roughly 60 to 70 per cent when applied in a droplet or in paste and approximately 95 per cent when applied by the beads, where the amount applied at once was much less than in the two other cases. It cannot be concluded from our experiments whether the decarboxylation occurred at the surface or inside the root. But the fact that most decarboxylation was found when the auxin supply was the least abrupt might point to the existence of sites of IAA decarboxylation at the root surface, where exoenzymes may be present. The chance of an auxin molecule to pass these enzymes would be greater if the number of molecules applied at once was larger. Recently KENDALL *et al.* (1971) also supposed that decarboxylation of IAA-^{14}C in a solution in which intact, sterile pea roots were submerged, occurred on the external surface of the roots. A possible role of epiphytic bacteria in the auxin decarboxylation cannot be judged from our results. Kendall *et al.* found a considerable $^{14}CO_2$ production with sterile roots, and we observed that in experiments with detached pea roots in solutions of IAA-^{14}C, the presence of 15 mg/l penicillin and streptomycin, which prevented bacterial growth, did not influence the $^{14}CO_2$ production. Their role, therefore, does not seem important.

In our 2-hours experiments the IAA-^{14}C was still present as such. This finding agrees well with the results of ANDREAE (1967), who did not find any formation

of indole-aspartate within 2 hours after application of IAA to pea root tissue. Summarizing, the fate of IAA-^{14}C following its application to intact pea roots was that only a relatively small portion of it in fact entered the roots, because most became decarboxylated. Moreover, the majority of this auxin became bound, so that only a minor part was detected as free IAA, probably present in the phloem. The transport of the IAA-^{14}C in the roots was predominantly in the acropetal direction.

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