Transport of Polyamines in Sugar Beet Seedlings

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The high levels of polyamines generally found in cotyledons of seedlings might be exported to hypocotyls and radicles. ^{14}C-Labelled putrescine applied to a sugar beet seedling through a cotyledon was found in small amounts as putrescine in the hypocotyl or radicle within a few minutes. Significant amounts of the labelled putrescine, however, were metabolized rapidly to compounds which could not be extracted into the organic phase on dansylation.

Introduction

There is evidence to support the involvement of polyamines in plant growth and development [1, 2]. While some authors suggest that polyamines may act as secondary messengers, others propose a hormone-like function. Uptake and transport studies are necessary to determine whether polyamines are acting as hormones or secondary messengers, or, are multifunctional cations. A strong argument against transport of polyamines out of cells is the presence of polyamine oxidases in cell walls [3–5]. Very little transport of putrescine and spermidine to shoots and roots was found 4 h after injection of ^{14}C-labelled amino acids and polyamines into cotyledons of etiolated pea seedlings [6]. An incubation time of 4 h is probably too long, because the bulk flow in the phloem is very rapid, with velocities of 50–200 cm h^{-1} [7]. Therefore, the incubation time for such experiments should be only a few minutes to avoid metabolism. In other experiments, long-distance translocation of polyamines was found and considerable amounts of polyamines were detected in vascular exudates [8]. Uptake of polyamines into cells occurs even against a concentration gradient [9–12].

High levels of polyamine synthesis activity can be found in cotyledons [13] where polyamines cannot contribute to any of their potential functions namely growth stimulation or regulation. Later in development, spermine and spermidine diminish in the cotyledons and increase in the shoots especially the epicotyls [14]. Polyamines could be exported from cotyledons into other parts of the plant, where they are probably needed, although often only in minute amounts. In the present study we show that putrescine is indeed transported. Samples for polyamine analysis were withdrawn from hypocotyls and radicles already a few minutes rather than several hours after injection of labelled putrescine into cotyledons.

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Materials and Methods

Growth conditions

Seeds of sugar beet (*Beta vulgaris* L. cv. Kawecora) were germinated on vermiculite at 22 °C with a 14 h light period (light intensity 30 μE $m^{-2}s^{-1}$) for 5 or 9 days.

Application of ^{14}C-labelled putrescine

One sugar beet cotyledon was cut 2 mm from the tip with a razor blade. The cotyledon was immediately dipped into 30 μl [1,4-^{14}C]putrescine·2 HCl (118 mCi $mmol^{-1}$, 50 μCi ml^{-1}; Amersham, England) adjusted to pH 5, 7.5 or 8 with 1 mM NaOH or 1 mM HCl. The putrescine solution was in a small container (4 mm tip of an Eppendorf tube). For $^{14}CO_2$ release measurements the plantlet and solution were covered with a plastic vial pierced by a pin with two pieces of filter-paper (15 mm diameter) impregnated with 50 μl 2 M KOH. At the appropriate time, seedlings were dissected into cotyledons, hypocotyl and radicle. The tissue was homogenized in a mortar containing 200 μl of a solution with 24% (w/v) perchloric acid, 4 mM unlabelled putrescine, 4 mM spermidine and 4 mM spermine, 400 μl dansylchloride (5 mg ml^{-1} acetone, freshly prepared) and 200 μl saturated sodium carbonate solution. The mixture was vortexed for 10 s and incubated in the dark for 16 h at room temperature. Unreacted dansylchloride was removed by adding 100 μl 0.87 M proline and incubated for 30 min. Dansylated amines

were extracted with 0.25 ml benzene, 200 µl was applied to thin layer plates (10 × 20 cm, Silica 60 F_{254} with concentration zone, Merck, Darmstadt, F.R.G.) and the plates developed with chloroform and triethylamine (5:1, v/v) as solvents. R_f values for putrescine and acetylputrescine were 0.53, and 0.20, respectively. Dansylated amines were visualized under UV light (254 nm), scraped and transferred into scintillation vials. The radioactivity was determined in 10 ml scintillation fluid (Hydrocount™, J. T. Baker Chemicals, B.V., Deventer, Netherlands) using a Tricarb 2000 CA counter (Packard, Canberra, Australia). Filter-papers used for $^{14}CO_2$ trapping were transferred into 10 ml scintillation fluid together with 1 ml 1 M HCl.

Results

Endogenous levels of putrescine, spermidine and spermine in the cotyledons, hypocotyl and radicles of 5- and 9-day-old sugar beet seedlings are presented in Table I. The highest amount of putrescine and spermidine was found in cotyledons of 5-day-old seedlings. A decreasing concentration gradient was found from cotyledons to hypocotyls to radicles. Older seedlings (9 days) contained much less putrescine and spermidine in the cotyledons than younger cotyledons. Passive diffusion of labelled putrescine from an external 0.424 mM putrescine solution into cotyledons can be excluded, because the application was carried out with 5-day-old seedlings, in which the cotyledons contained 896 nmol putrescine per gram fresh weight.

Table I. Levels of putrescine (put), spermidine (spd) and spermine (spn) in 5 or 9 days old sugar beet seedlings in nmol per gram fresh weight (nd, not detectable). Cadaverine was not found.

Organ	5 day old			9 day old		
	put	spd	spn	put	spd	spn
Cotyledons	896	1157	492	104	70	353
Hypocotyl	53	231	598	20	1	406
Radicle	48	86	466	25	nd	249

After removal of 2 mm of the tip of one cotyledon and dipping it into a solution with labelled putrescine, the distribution of labelled putrescine over the seedling was determined. As effects of pH could be important [10], the optimal pH for putrescine uptake and transport was determined by adjusting the external pH to 5, 7.5 and 8 with HCl or NaOH. After 3 h radicles contained 24,200 dpm, 3650 dpm and 14,200 dpm of radiolabelled material at pH 8, 7.5, and 5, respectively. This gross examination confirmed published results [10], where two pH maxima for putrescine uptake into Saintpaulia petals were found. Further experiments were carried out at pH 8.

In preliminary experiments carried out over a period of 4 h, movement of labelled putrescine in the seedling could not be detected (results not shown). An incubation time of 4 h was probably too long, because the bulk flow in the phloem can be very rapid, with velocities of 50–200 cm h^{-1} [7]. Therefore, uptake and transport assays were done over a period of minutes rather than hours.

After 60 min 23% of the radioactivity was found in the plantlets (Table II). Almost all radioactivity

Table II. Radioactivity in the seedling after extraction in water phases and benzene phases (values for dansylated amines obtained from the cotyledons, the hypocotyl and radicle summed up), radioactivity left in the solution applied and radioactivity released as $^{14}CO_2$ (average of two seedlings; nd, not determined). 15 µCi were supplied to each seedling.

Time [min]	Radiolabelled material (dpm × 10^{-2})* Seedling fraction in water phase	fraction in benzene phase	Left-over in solution applied	$^{14}CO_2$ release	Total
5	711	51	29850	nd	30612
10	1069	66	28837	nd	29972
20	3621	257	25639	nd	29517
40	4175	418	25420	nd	30013
60	6283	598	22929	10	29820

* The dpm data have been multiplied with 10^{-2}.

(around 90%) was in the water phase obtained after extraction of dansylated amines with benzene. This radioactive material must consist of metabolites of putrescine, which could not be dansylated, because control experiments showed that 85% of putrescine in a water solution was dansylated during our procedure and only 15% of the radioactivity was found in the water phase, representing unreacted putrescine and impurities. In extracts from all parts of the plantlet an increase of radioactivity in the water phase after extraction of dansylated amines with benzene was observed over time, however, most of the labelled putrescine was metabolized at the site of application, *i.e.* the cotyledon (Fig. 1). Only a little putrescine was metabolized to CO_2 (Table II). Most of the labelled putrescine, together with another compound, did not move from the application site to the other plant parts analyzed (Fig. 2); lower amounts of putrescine were translocated from the application site to the untreated cotyledon (Fig. 2b), the hypocotyl (Fig. 2c) or the radicle (Fig. 2d). Measurable amounts of spermidine and spermine (610 and 360 dpm resp. after 60 min) were formed at the application site only. It is interesting to note, that putrescine was always accompanied by another compound (Fig. 2); the analysis on silica thin layer plates showed that the compound has the same R_f value as acetylputrescine (0.20).

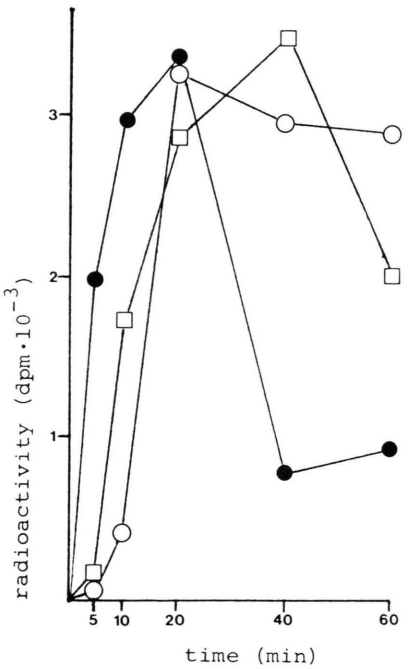

Fig. 1. Radioactive material found in the water phase after extraction of dansylated amines with benzene from different plant parts (●, untreated cotyledon; □, hypocotyl; ○, radicle). In the cotyledon, where labelled putrescine was introduced, radioactivity increased to 622,400 dpm almost linearly within 60 min (data not shown).

Discussion

The data presented by Young and Galston [6] using etiolated pea seedlings showed total radioactivity found in different parts of the seedling after 4 h. The low transport activity in pea seedlings is not surprising, because it is directed against a polyamine concentration gradient (from cotyledon to radicle). As pea and soybean seedlings may be the exception rather than the rule in showing a reversed gradient [13], they are unsuitable as general model systems for transport studies. Normally, cotyledons synthesize polyamines during germination and metabolize or excrete them later to other parts of the seedling (Table I, [13]). After treatment of one sugar beet cotyledon with labelled putrescine, small amounts of labelled putrescine and "acetylputrescine" were found in the other parts of the seedlings (Fig. 2). It is difficult to determine the amount of putrescine transported in sugar beet seedlings, because the dilution by the unlabelled putrescine already present in the cotyledons, hypocotyl and radicle and extensive degradation of putrescine at the application site, during transport and at the target site (Fig. 1) complicate the interpretation.

The high levels of polyamine synthesis generally found in the cotyledons of germinating seeds cannot be fully explained by the association of polyamines with rapidly dividing cells [13]. Polyamines either could be used in the cotyledon, *e.g.* for unfolding, or they could be exported to other parts of the seedling. In our experiments, radiolabelled putrescine and another compound, possibly acetylputrescine, are present in both the hypocotyl and radicle in small amounts, already a few minutes after application to one cotyledon. The interval would be too short for extensive metabolism of labelled putrescine into some transport forms and subsequent reformation of putrescine in particular parts of the seedling.

A high level of transport is not necessary to satisfy the nutritional needs of a seedling, because the

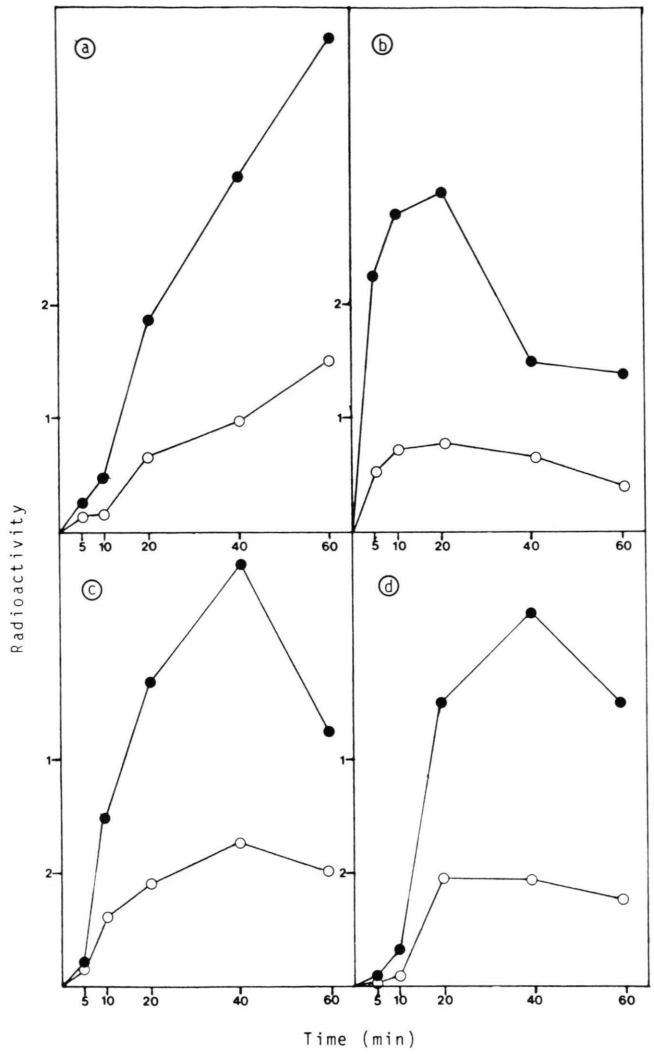

Fig. 2. Uptake and transport of labelled putrescine into different parts of a sugar beet seedling. The amines in the extracts were dansylated, extracted with benzene and separated by thin-layer chromatography. Both cotyledons, the one, where labelled putrescine was applied (a), the untreated cotyledon (b), the hypocotyl (c) and the radicle (d) were analyzed for radiolabelled material (●, "acetylputrescine", ○, putrescine). In Fig. 1a 1 unit of radioactivity corresponds to 10^4 dpm, in Fig. 1b, 1c and 1d to 60 dpm.

amount of polyamines found in hypocotyls and radicles of different plant species is frequently very low [13]. Nevertheless, it cannot be excluded, that polyamines in cotyledons are simply a waste product or a nitrogen source. The metabolites of putrescine found after the dansylation procedure in the water phase (Fig. 1) could be important, because they may play a role explaining the function of polyamines in cotyledons.

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[1] K. D. Slocum, R. Kaur-Sawhney, and A. W. Galston, Arch. Biochem. Biophys. **235,** 283–303 (1984).
[2] T. A. Smith, Annu. Rev. Plant Physiol. **36,** 117–143 (1985).
[3] R. Federico and R. Angelini, Planta **167,** 300–302 (1986).
[4] R. Kaur-Sawhney, H. E. Flores, and A. W. Galston, Plant Physiol. **68,** 494–498 (1981).
[5] I. Sinska, Wiad. Bot. **30,** 9–24 (1986).
[6] N. D. Young and A. W. Galston, Plant Physiol. **73,** 912–914 (1983).
[7] J. Burgess, An introduction to plant cell development, p. 100, Cambridge Univ. Press, Cambridge 1985.
[8] R. Friedman, N. Levin, and A. Altman, Plant Physiol. **82,** 1154–1157 (1986).
[9] N. Bagni, R. Baraldi, and G. Costa, Acta Hortic. **149,** 173–178 (1984).
[10] N. Bagni and R. Pistocchi, Plant Physiol. **77,** 398–402 (1985).
[11] R. Pistocchi, N. Bagni, and J. A. Creus, Plant Physiol. **80,** 556–560 (1986).
[12] R. Pistocchi, N. Bagni, and J. A. Creus, Plant Physiol. **84,** 374–380 (1987).
[13] H. Felix and J. Harr, Physiol. Plant. **71,** 245–250 (1987).
[14] N. Bagni, New Phytol. **69,** 159–164 (1970).