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REVIEW

Polyamine uptake and translocation in plants

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Abstract

Recently, evidence has increased for both long- and short-distance transport of polyamines (PAs) in living organisms, but the mechanisms involved and physiological significance of PAs translocation are still not well understood. This review deals with various aspects of polyamine uptake and transport in higher plant tissues.

Additional key words: putrescine, spermine, spermidine, transport.

Introduction

Polyamines (PAs) are ubiquitous polycationic nitrogenous bases reported to have several important functions in living organisms including microorganisms, plants and animals (for reviews, see, Evans and Malmberg 1989, Galston and Kaur-Sawhney 1990, Smith 1990, Kakkar and Rai 1993, 1997). They modulate several processes related to growth and development (cell division, differentiation, embryogenesis, rhizogenesis, senescence, flowering, fruit ripening), and are involved in stress responses (Evans and Malmberg 1989). In all these processes, PAs have been ascribed various roles such as that of a new class of plant growth regulators,

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Abbreviations: CCCP - carbonylcyanide-*m*-chlorophenylhydrazone; CHA - cyclohexylamine; DCCD - dicyclohexylcarbodiimide; DES - diethylstilbestrol; DFMO - difluoromethyl ornithine; 2,4-DNP - 2,4-dinitrophenol; EGTA - ethylene glyco-bis[β-aminoethyl ether]N,N,N,N-tetra acetic acid; Em - membrane potential, FCCP - carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; MGBG - methylglyoxal-bis(guanylhydrazone); NEM - N-ethylmaleimide; ODC - ornithine decarboxylase; PCMBS - *p*-chloromercuribenzene sulphonic acid; PA(s) - polyamine(s); Put - putrescine; Spd - spermidine; Spm - spermine.

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hormonal second-messengers and as one of the reserves of carbon and nitrogen at least in cultured tissues (Flores and Filner 1985, Slocum and Flores 1991). PAs can stimulate ribosome subunit association, stabilize the t-RNA structure and reduce the rate of RNA degradation, enhance both RNA and DNA synthesis, help to condense DNA, covalently modify proteins as well as regulate the rigidity and stability of cellular membranes (Janne *et al.* 1978, Tabor and Tabor 1984, Roberts *et al.* 1986, Pegg 1986, Kaur-Sawhney and Applewhite 1993).

Most of the living cells including plants can carry out *de novo* synthesis of PAs but the physiological significance of PA transport from the application zone and/or from their site of synthesis to the target tissues in plants still remains unclear (Galston and Kaur-Sawhney 1990, Bagni and Pistocchi 1992). Can we consider PAs as phytohormones because of their mobility, since the latter is an important criterion for their categorization in the class of phytohormones? Do PAs, like phytohormones, have a regulatory role in long-distance transport? Does PA partitioning have some physiological significance or are cytoplasmic PA levels regulated by compartmentation, conjugation, degradation and/or interaction with cell wall constituents? The present article deals with all these unanswered questions and also includes mechanisms, kinetics, and physiological significance of PA uptake and translocation in the plant systems.

General characteristics of polyamine uptake

The mechanism(s) and kinetics of PA uptake has been studied both at cellular and subcellular levels in various plants, animals and micro-organisms (Table 1). Uptake of PA and/or difluoromethyl ornithine (DFMO)–a specific irreversible inhibitor of ornithine decarboxylase (ODC) seems almost similar to amino acid transport (for review see Frommer *et al.* 1994). In the petals of *Saintpaulia ionantha* (African violet), putrescine (Put) uptake occurred at a low (0.5 μM - 0.1 mM, pH 5.0 - 5.5) concentration gradient, and followed at higher concentration gradient (0.1 - 100 mM, pH 8.0). Uptake rates were constant for 2 h and reached a maximum after 3 to 4 h (Bagni and Pistocchi 1985). The Km was 8.63 mM (pH 5.5) which was considerably higher than the Km (between 1.1 and 1.5 μM) for Put uptake in animals, depending on the species and tissue, and was regulated by a saturable protein-mediated transport system. However, the authors later argued that the use of *Saintpaulia* petals represented a mature and differentiated system, and was not suitable for studies on growth substances (Bagni and Pistocchi 1990). In the lichen *Evernia prunastri*, uptake of PAs was also pH dependent (Escribano and Legaz 1985). In carrot cell suspensions, uptake of Put was rapid, reaching a saturation after 1 - 2 min. The concentration-dependent uptake of Put was biphasic between 1 μM and 100 mM and could be resolved into two saturable components with Km values of 41.9 μM and 29.2 mM (Pistocchi *et al.* 1987). In another study, using carrot protoplasts and vacuoles, rapid accumulation of PAs, with maximum absorption within 1 - 2 min has also been found (Pistocchi *et al.* 1988). Spermidine (Spd) uptake (between

pH 5.5 - 7.0) was linear up to the highest concentration tested in protoplasts, while that in vacuoles (optimum pH 7.0) showed saturation below 1 mM (Km = 61.8 μM) and a linear component from 1 to 50 mM. Similarly, uptake of Spd was rapid in leaf tissue of cowpea (*Vigna unguiculata* L.), while a 20-h lag period was necessary before the onset of Spd uptake in cowpea protoplasts (Joshi *et al.* 1983). In maize cell lines, all extracellular Put was taken up within 8 min (Hiatt 1989). ^{14}C-Put loaded into the maize roots for 24 h and accumulated in the vacuole was capable of moving back out of the vacuole across the tonoplast and the plasmalemma (Di-Tomaso *et al.* 1992a,b). They proposed that endogenously synthesized Put could be transported across plant membranes into the apoplasm and subsequently via the xylem to other tissues or organs. In another study, uptake of ^{14}C-Spd increased rapidly up to 1 h, thereafter remained constant up to 4 h, by the time 18 % of the label had been absorbed in excised soybean leaves (Caffaro *et al.* 1993).

Table 1. Km values for polyamine (putrescine, spermidine or spermine) uptake in plants in systems I and II (see text for details).

Material	Polyamine	System I	System II	References
Saintpaulia petals	Put	8.6 mM (pH 5.5)		Bagni and Pistocchi 1985
		2.4 mM (pH 8.0)		
	Spd	1.2 mM		
	Spm	2.1 mM		
Carrot cell cultures	Put	41.9 μM	29.2 mM	Pistocchi *et al.* 1987
	Spd	27.3 mM	linear	
	Spm	7.7 mM	15.9 mM	
Carrot protoplasts	Spd	linear (pH 5.5-7.0)	linear	Pistocchi *et al.* 1988
	Spm	292.0 μM	linear	
	Spm + Ca^{2+}	122.0 μM	linear	
Carrot vacuoles	Spd	61.8 μM (pH 7.0)	linear	
Helianthus tuberosus mitochondria	Spd	89.0 μM		Pistocchi *et al.* 1990
Maize seedling roots	Put	1.2 μM		Di-Tomaso *et al.* 1992b
Porphyridium sp.	Put	linear	linear	Scoccianti *et al.* 1989
	Spm	0.3 mM		
	Spd	1.2 mM	6.7 mM	

In unicellular red alga, *Porphyridium* sp., Put uptake was linear up to the highest concentration tested (100 mM), while other two PAs gave saturation kinetic curves with Km values of 0.3 and 1.6 mM up to 4.0 mM (system I) and of Spd with Km value of 6.7 mM up to 50 mM (system II) (Scoccianti and Bagni 1992). The involvement of diffusion in efflux of PAs, probably through ionic channels, was demonstrated in *Arabidopsis thaliana* cells (Colombo *et al.* 1992). Badini *et al.* (1994) reported accumulation of PA in the seaweed, *Ulva rigida* following

concentration gradient and displayed linear kinetics. Uptake was passive as metabolic inhibitors could not block transport.

Uptake of DFMO by roots of barley seedling was biphasic with Km values of 1.6 mM and 53.3 mM for system I and II, respectively. Uptake was saturable and substantially reduced by ornithine (Walters and Kingham 1990). Uptake of CHA (competitive and reversible inhibitor of Spd synthase) in excised cotyledons of radiata pine (*Pinus radiata*), was linear between 0.5 and 20 nM. CHA was rapidly absorbed within 6 h followed by decline between 6 and 24 h and occurred against a concentration gradient. In fact, the internal concentration exceeded the external one by several fold, reaching a cellular concentration of 5.1 mM after 48 h in a medium supplied with 1mM CHA. Cotyledons cultured on benzylamine-containing medium accumulated more CHA than those in benzylamine-free medium (Biondi *et al.* 1986). Similary in carrot cells, internal concentration of DFMO was 1000-fold higher than the external DFMO concentration (Mengoli *et al.* 1989).

Short-and long-distance transport of polyamines in plant tissues

On the bases of studies of short- and long-distance transport of PAs in intact and excised plant tissues it is supposed that PAs belong to the group of phytohormones rather than to second-messengers. In apple, [^{3}H]-Put was absorbed by the leaves and translocated to fruits and *vice versa* (Bagni *et al.* 1984). The translocation occurred via peduncle and was not polar. On the contrary, translocation of cadaverine in *Nicotiana glauca* was only from the roots to leaves (Bagni *et al.* 1986). Cadaverine has also been reported to be present in shoots (Yokota *et al.* 1994), grains (Umezu 1961) and germ (Moruzzi and Caldarera 1964) of rice, while etiolated pea seedlings supplied with labelled Put and Spd exported little of these molecules to shoots and roots, 4 h after the injection (Young and Galston 1983). Possibly, these PAs might have been degraded before they could reach the transport site, since diamine oxidase activity is known to be very active in legumes (Smith and Barker 1988).

The physiological significance of PA transport using indirect approaches, in different plants, has also been reported. Exogenous PAs increased fruit growth, fruit set and yield per tree in apple (Costa and Bagni 1983). Similarly, the localized and differential effects on chlorophyll loss following exogenous application of PAs to soybean leaves was observed (Cheng and Kao 1983). Also, exogenously applied, Put, Spd and Spm (0.01 M each) to cut stems of tomato reduced ozone damage to leaves and thus indirectly supporting the possibility of intercellular transport (Ormrod and Beckerson 1986). A rapid translocation of labelled Put through the funiculus and via suspensor to the growing embryo of *Phaseolus coccineus* indicated PA requirement for embryogenesis and fruit development (Nagl 1990). The translocation of PAs from leaves to the axillary and apical buds in soybean (Caffaro *et al.* 1994) has been considered as part of the complex mechanism of the flowering signal and their involvement during transition of vegetative to flowering buds.

The long-distance translocation of PAs have been studied physiologically by analysing the constituents of phloem and xylem sap. High concentrations of Put and

Spd were found in xylem exudates of sunflower, mungbean and orange stems while grapevine had lower concentrations. Spm was found only in trace amounts in exudates of all species. Put concentration was higher in exudates of older than in younger sunflower plants and also of salt-stressed plants. Put and Spd were also found in the phloem sap of sunflower and mungbean plants (Friedman *et al.* 1986). In another study, [^{3}H]-Put translocation in the upper parts (cotyledons and coleoptiles) of the seedlings of tomato, maize and pine occurred mainly through xylem vessels. This long-distance transport was not affected in pine seedlings that were ringed to exclude cortical parenchyma and phloem (Rabiti *et al.* 1989). The transport was dependent on temperature, relative humidity and rate of transpiration. In yet another study, foliar sprays of Put or benzyladenine on potato increased the transport of ^{86}Rb to the apex, whereas Spd or ABA induced translocation into the growing potato tuber. ^{14}C-Put supplied to a leaf was transported via the phloem and movement was bi-directional. These effects were correlated to the distribution of endogenous PAs within the plant (Feray *et al.* 1992). However, in soybean the direction of translocation of [^{14}C]-Spd applied to the central leaflet was not polar, as radioactivity derived from Spd was found both in the apical bud and in roots. The rate of translocation was higher in the basipetal (via phloem and cortical parenchyma) than in the acropetal (via xylem) direction (Caffaro *et al.* 1993). It was observed recently, that Put exported through phloem from induced mature leaves to the apex might control cell division cycle and hence regulate flowering in *Sinapis alba* in this way (Havelange *et al.* 1996). The extra Put synthesized in induced leaves is suggested to be a necessary component of the floral stimulus.

In sugar beet seedlings, [^{14}C] Put applied to cotyledons, was exported to the hypocotyl and radicle within a few minutes and was rapidly metabolized (Christ *et al.* 1989). A different approach to verify the occurrence of PA transport was that of Masse *et al.* (1989) who studied uptake and translocation of a synthetic analogue of Spm (N,N'-bis[3-aminopropylamino]ethane during the *in vitro* growth of potato and maize plantlets. Uptake of the tetramine was concentration-dependent and a degradation product was also found in both roots and shoots of the treated plants. On the other hand, exposure of maize roots to 5 mM Put caused a rapid depolarization of the membrane potential (Em) within 1 min, complete inhibition in net K^{+} flux across the plasma membrane within 15 min and death of the root within 24 h (Di-Tomaso *et al.* 1989). DFMO also appeared to possess some phloem mobility and can be transported from shoot to root and from lower to upper leaves in barley (Walters and Kingham 1990).

Subcellular localization of polyamines in plants

Little information exists for the subcellular localization of PAs in plants. Being flexible polycations, they can readily associate with any anionic macromolecules. However, almost all the PAs have been found mainly in the cell wall fraction and vacuoles, the later represent a storage site for the PAs. Goldberg and Perdrizet (1984) showed that endogenous PAs are present in the cell wall in mungbean hypocotyls,

whereas, in *Neurospora*, 28 % of the cellular Spd was vacuolar and the remainder was strongly bound to anionic sites in the cell (Paulus *et al.* 1983). Walker *et al.* (1987) found nearly all of the free endogenous Put and Spd in the supernatant fraction of two cell lines of *Nicotiana tabacum* as well as their biosynthetic enzymes. Pistocchi *et al.* (1988) showed the presence of 42 and 28 % of Put and Spd, respectively, in the carrot vacuoles. PAs and their biosynthetic enzymes have also been reported to be present in mitochondria and chloroplasts (Torrigiani *et al.* 1986) and this compartmentation has been attributed to the activity of the biosynthetic enzymes found in these organelles. PAs are also present in nuclei but Walker *et al.* (1987) did not find substantial amounts of Put and Spd in this fraction.

Competition experiments

Most, if not all studies have indicated enhancement of PA uptake in the presence of Ca^{2+}. Two functions for Ca^{2+} in inducing PA uptake have been suggested. Firstly, it might help in the maintenance of membrane integrity, and secondly its possible role in the maintenance of transport system(s) which appeared to be highly specific by activating ionic channels, ATPases or other enzymes. Like amino acids, PA uptake and translocation may also occur via multiple transport system with different specificities and transport modes, however, uptake mediated through a broad specificity transport system which shows no or little competition has also been reported. In petals of *Saintpaulia*, Put and Spd uptake was stimulated by Ca^{2+}, Mg^{2+}, and K^+ at equimolar concentration (17 μM each) (Pistocchi *et al.* 1986). Similarly, in carrot cell cultures, Ca^{2+} stimulated Put uptake by 35 % at 1 μM concentration but inhibited uptake at concentrations between 50 μM and 1 mM (Pistocchi *et al.* 1987). In carrot protoplasts, uptake of Spm (100 μM - 1 mM) was enhanced by 1 mM Ca^{2+}. Ca^{2+} affected the Km which decreased from 292 to 122 μM. Since the uptake value was very low in the absence of Ca^{2+} this cation seemed to be necessary for PA transport in protoplasts (Pistocchi *et al.* 1988). In the presence of Ca^{2+}, 0.5 mM Put had a slight stimulatory effect on the net flux of K^+ in the roots of maize seedlings. However, when Ca^{2+} were chelated from the root tissue with EGTA, exposure to 0.5 mM external Put resulted in a depolarization of the Em, reduction in the K^+-induced depolarization of Em and a dramatic alteration in K^+ transport from influx to a substantial efflux. Thus it is suggested that Put could not replace Ca^{2+} in maintaining membrane stability (Di-Tomaso *et al.* 1989). In carrot protoplasts, in the absence of Ca^{2+}, Put transport was inhibited by Spm while Spd uptake was inhibited by Spd and Spm, indicating that transport system was shared by all the PAs. However, in the presence of Ca^{2+} Spm (100 μM) stimulated both Put and Spd uptake suggesting separate and distinct transport systems for different PAs (Antognoni *et al.* 1993). La^{3+} (an impermeant cation and competitive inhibitor of Ca^{2+} transport) negated the stimulatory effect of Ca^{2+} on Put uptake whereas, it mimicked the stimulatory effect of Ca^{2+} on Spd and Spm uptake at concentrations 100 μM and 1 mM, respectively (Pistocchi *et al.* 1987). In carrot protoplasts, La^{3+} up to 1 mM

stimulated Spm uptake by four fold while Mg^{2+} (10 μM to 10 mM) had no effect on uptake either in cell cultures or protoplasts (Pistocchi and Bagni 1990). These authors also observed that the presence of acetyl-putrescine or -spermine, PA analogues having a reduced number of positive charges did not affect the uptake of the corresponding labelled nonacetylated amine.

Monovalent cations such as Na^+ and K^+ at high concentrations (>50 mM) competitively inhibited the uptake of Put in carrot cell suspensions (De Agazio *et al.* 1989). In their earlier studies, authors reported reduction in K^+ uptake by Put, which was possibly due to the phytotoxic effect of the degradation products of Put oxidation, *e.g.* H_2O_2 and oxygen radicals (De Agazio *et al.* 1988). In mitochondria of *Helianthus tuberosus*, 0.2 - 1 mM Mg^{2+} reduced the amount of Spd absorbed as did 20 - 100 mM K^+, while Ca^{2+} had no effect on Spd uptake (Pistocchi *et al.* 1990). However, 10^{-7} to 10^{-4} M indole-3-acetic acid (IAA) enhanced [^{14}C]-Spd uptake in the presence of Ca^{2+} (1 mM) in carrot protoplasts, whereas 100 μM vanadate (an inhibitor of H^+-ATPase) inhibited Spd uptake thereby suggesting an energy-dependent transport (Kanchanapoon *et al.* 1991).

Various metabolic inhibitors have also been tested to check whether the transport of PAs was energy-dependent. Using *Saintpaulia* petals, uncouplers such as 2,4-dinitrophenol (2,4-DNP) and carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) (0.01 and 0.1mM concentration each) had no effect on putrescine uptake, however, inhibition of Spd uptake was obtained both with 20 mM NaSCN (68 %) and low temperature 0 °C (35 %) (Pistocchi *et al.* 1986). While in carrot cells, 0.1 mM CCCP and 1 mM 2,4-DNP inhibited Spd and Put uptake by 28 and 34 %, respectively, whereas dicyclohexylcarbodiimide (DCCD) and diethylstilbestrol (DES) (inhibitors of oxidative phosphorylation), vanadate and N-ethylmaleimide (NEM; impermeant sulfhydryl reagent) were ineffective both in the presence or absence of Ca^{2+}. The low percentage of inhibition might be due to the interactions between the inhibitors and some cell wall constituents (Pistocchi *et al.* 1987). In carrot protoplasts, Spd and Spm uptake was inhibited by 50 % by 10 μM carbonyl cyanide-*p*-trifluoromethoxy-phenylhydrazone (FCCP) only in the presence of 1 mM Ca^{2+}, while it was not affected in the presence of 1 mM La^{3+} or Spm alone. Thus, it appeared that the Ca^{2+}-stimulated uptake was an energy-dependent mechanism and that the function of Ca^{2+} was more specific than that of La^{3+} (Pistocchi *et al.* 1988).

In mitochondria of *Helianthus tuberosus*, antimycin-A (electron transport inhibitor) completely blocked the uptake of [^{14}C]-Spd. Furthermore, ionophores such as valinomycin (10 μM) and nigericin (100 μM) completely and slightly inhibited movement of Spd, respectively. FCCP, which causes the suppression of the total electrochemical gradient inhibited Spd uptake (Pistocchi *et al.* 1990). In maize seedlings, sulfhydryl reagents, such as NEM at 0.3 mM concentration had no effect on Put uptake while PCMBS (an impermeant) at 2 mM concentration only partially inhibited transport of the diamine (39 % inhibition). CCCP (20 μM) and KCN (0.5 mM) also partially inhibited the saturable component of Put uptake. This inhibition has been correlated to depolarization of Em (Di-Tomaso *et al.* 1992b).

In maize roots, cadaverine and the herbicide paraquat (a bipyridinium quaternary salt) competitively inhibited Put uptake whereas, Spm appeared to be a non-competitive inhibitor (Hart *et al.* 1992, Di-Tomaso *et al.* 1992b). This competitive inhibition could possibly be due to a similar charge distribution between two amino groups for the two divalent cationic compounds such as Put and Cad (Schuber 1989). On the contrary, paraquat did not affect the uptake of Put in carrot protoplasts (Antognoni *et al.* 1993). However, in maize seedlings Put uptake was non-competitively inhibited by other polyvalent cations including Ca^{2+} (50 μM - 5 mM), Mg^{2+} (1.8 mM), La^{3+} (200 μM) and Spm, which did not share similar charge distribution. This non-competitive effect might possibly be due to charge shielding, binding to the Put transport protein or to the interaction with the lipid bilayer (Di-Tomaso *et al.* 1992b).

Conclusions and future perspectives

In bacterial and animal systems, it has been known for many years that exogenous PAs are transported and accumulated by cells, however in plants the translocation of PAs has been shown recently only and its mechanism is not yet well understood. The reason could be their ubiquity as almost all cells of different organs have different rates of PA synthesis and PA contents. Nevertheless, PA transport under physiological conditions may be necessary to regulate endogenous PA concentration and/or activity. Moreover, diamine and PA oxidases localized in the cell wall, might also regulate intercellular transport of PAs. Polyamines and enzymes for their biosynthesis are mainly localized in the cell wall, vacuoles, nuclei, mitochondria and chloroplasts and their compartmentation following uptake is important and has physiological significance. Also the hypothesis that vacuoles represent storage sites for PAs needs further research. It seems that compartmentation of PAs, their interaction with cell wall constituents, the presence of conjugated/bound PAs and diamine and PA oxidase activities might participate in the regulation of cytoplasmic levels of PAs. Do different plant species, their age as well as control by other plant hormones have any relevance in regulating the transport of PAs? Hormonal treatments are able to specifically change the partitioning of nutrients. Can PAs influence such type of reallocation? Further, whether PAs inhibitors and inorganic cations have different channels for uptake, is yet to be confirmed. Little is known about the molecular mechanisms by which Ca^{2+} affects the transport. At present, we are not even aware of existence of a single or different carriers for PAs (Put, Spd, Spm). Recently, combination of molecular tools with the analysis of mutants has allowed to get a first insight into the PA transport at least in microorganisms. Isolated protoplasts and/or cell organelles and plant mutants would be ideal systems to elucidate the mechanism of PA transport. Radio-labelled PAs and/or their precursors can also be utilized to study their translocation in excised tissue and/or intact seedlings. Translocation can also be studied using indirect approaches, like studying the effects of PAs and PA biosynthetic inhibitors on uptake in excised tissues as leaf discs, flower petals, *etc.* Although, some attempts have already been made in these

regards, the true physiological significance of translocation is yet to be elucidated. However, the presence of PAs in the vascular sap (xylem and phloem exudates) of plants is regarded as strong evidence for their translocation between organs.

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