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Biosynthesis, oxidation and conjugation of aliphatic polyamines in higher plants

Review Article

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Summary. This chapter will focus on polyamine biosynthesis, oxidation, conjugation processes, mainly to hydroxycinnamic acids, and compartmentation of enzymes, substrates and products, giving an overview about recent results especially in higher plants. New research advances regarding the cloning of the main cDNA encoding for polyamine biosynthetic and oxidative enzymes, will be taken into consideration.

Keywords: Amino acids – Arginine – Ornithine – Putrescine – Spermidine – Spermine – Polyamines

Abbreviations: AIH, agmatine iminohydrolase; ARS, argininosuccinate; DAO, diamine oxidase; GABA, 4-amino-n-butyric-acid; NCPAH, N-carbamoylputrescine amidohydrolase; ODC, ornithine decarboxylase; PAO, polyamine oxidase; PCA, perchloric acid; SAM, S-adenosylmethionine; SAMDC, S-adenosylmethionine decarboxylase

Introduction

Natural occurring amines, derived from ammonia by substitution, can be divided into primary, secondary, tertiary and quaternary. A large number of these amines have been identified in higher plants. They could derive from aminoacids decarboxylation or from aldehydes transamination that is an important source of simple aliphatic amines.

Among the primary aliphatic amines, widely distributed throughout the plant kingdom, small monoamines are often produced in flowers at anthesis or in fruiting bodies of fungi. These amines (Table 1), by simulating rotting meat smell, play an important role in attracting insects which contribute to fecondation by carrying pollens or spores. Similarly, in *Araceae* family several aliphatic amines are produced and released in the surroundings as consequence

Table 1. Aliphatic monoamines in plants

Trivial name	Chemical structure
Methylamine	CH_3NH_2
Dimethylamine	$(CH_3)_2NH$
Trimethylamine	$(CH_3)_3N$
Ethylamine	$CH_3CH_2NH_2$
Diethylamine	$CH_3CH_2NHCH_3CH_2$
n-Propylamine	$CH_3(CH_2)_2NH_2$
n-Buthylamine	$CH_3(CH_2)_3NH_2$
Amylamine	$CH_3(CH_2)_4NH_2$
n-Hexylamine	$CH_3(CH_2)_5NH_2$
Ethanolamine	$OH(CH_2)_2NH_2$
Propanolamine	$OH(CH_2)_3NH_2$

of a temperature increase within the odoriferous inflorescence (Smith and Meeuse, 1966). It's important to underline that the widespread monoamines, methylamine and ethanolmine (Table 1), sometimes are also obtained as extraction artefacts from the degradation of phosphatides (Hartmann et al., 1972).

Aliphatic polyamines, ubiquitous compounds classified as growth substances (Bagni, 1989), have been implicated in a large range of plant growth and developmental processes such as cell division (Bagni, 1989), embryogenesis, morphogenesis (Bagni et al., 1993a) and response to environmental stresses (Bouchereau et al., 1999), even if their precise physiological function and mechanism of action still remain unclear. The studies on polyamine function were highly complicated by the lack of plant mutants deficient in polyamines. A great part of the information available about polyamine biosynthetic pathways were obtained by using a wide range of metabolic inhibitors (Tiburcio et al., 1990) and more recently by cloning some of the genes encoding for polyamine biosynthetic and oxidative enzymes (Chattopadhyay and Ghost, 1998; Tavladoraki et al., 1998). Moreover, numerous papers have been published in the last years regarding transport, compartmentation and binding of polyamines, providing further detailed information about polyamine action (Bagni et al., 1993b).

Other important compounds, which will not be taken into consideration in this context, are aromatic amines, some of them very important for pharmacological studies, and secondary amines, which, by reacting with nitrous acid may give carcinogenic nitrosamines, such as dimethylnitrosamine [$(CH_3)_2N - N = 0$] which is present in *Solanum incanum* (Du Plessis et al., 1969) eaten by Bantù people in Transkein region (South Africa).

Aliphatic polyamines

Aliphatic polyamines are synthesised in both prokaryotic and eukaryotic organisms. The most common ones are the diamine putrescine (1,4 diaminobutane), the triamine spermidine (1,8-diamino-4-azaoctane) and the

Table 2. Common and uncommon natural occurring aliphatic polyamines

Trivial name	Systematic name	Chemical structure
1,3-Diaminopropane	1,3-Diaminopropane	$NH_2(CH_2)_3NH_2$
Putrescine	1,4-Diaminobutane	$NH_2(CH_2)_4NH_2$
Norspermidine (caldine)	1,7-Diamino-4-azaheptane	$NH_2(CH_2)_3NH(CH_2)_3NH_2$
Spermidine	1,8-Diamino-4-azaoctane	$NH_2(CH_2)_3NH(CH_2)_4NH_2$
sym-Homospermidine	1,9-Diamino-5-azanonane	$NH_2(CH_2)_4NH(CH_2)_4NH_2$
Thermine	1,11-Diamino-4,8-diazaundecane	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$
Spermine	1,12-Diamino-4,9-diazadodecane	$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$
Thermospermine	1,12-Diamino-4,8-diazadodecane	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$
Homospermine	1,13-Diamino-4,9-diazatridecane	$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_4NH_2$
Caldopentamine	1,15-Diamino-4,8,12-triazapentadecane	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$
Homocaldopentamine	1,16-Diamino-4,8,12-triazahexadecane	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$
Homopentamine	1,19-Diamino-5,10,15-triazanonadecane	$NH_2(CH_2)_4NH(CH_2)_4NH(CH_2)_4NH(CH_2)_4NH_2$
Caldohexamine	1,19-Diamino-4,8,12,16-tetraazanonadecane	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3\ NH(CH_2)_3NH_2$
Homocaldohexamine	1,20-Diamino-4,8,12,16-tetraazaeicosane	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3\ NH(CH_2)_4NH_2$
N^4-Aminopropyl-norspermidine		$[NH_2(CH_2)_3]_3N$
N^4-Aminopropyl-spermidine		$[NH_2(CH_2)_3]_2N[NH_2(CH_2)_4]$

tetramine spermine (1,12-diamino-4,9-diazadodecane). Many other di- and polyamines are present in plants and microorganisms, such as the diamines 1,3-diaminopropane and cadaverine (1,5-diaminopentane) (Table 2). Unusual polyamines have also been detected in bacteria, algae, fungi, animals and higher plants (Niitsu and Samejima, 1993). In the extreme thermophilic bacteria *Thermus thermophilus* at least 14 polyamines, among which some linear and branched pentamines, hexamines and heptamines, have been isolated (Table 2). Caldopentamine was present in considerable amount especially in bacterial cells grown at extremely high temperatures (80°C or more) (Oshima, 1989). These unusual polyamines, typical and abundant in thermophilic bacteria, can be nevertheless considered widespread. They were in fact detected in some Leguminoseae, such as *Canavalia gladiata* and *Vicia radiata* (Matsuzaki et al., 1990; Hamana et al., 1991), leading to hypothesise a putative role of these molecules in growth and differentiation processes.

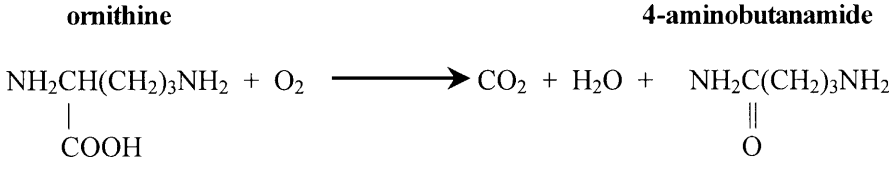
Among common polyamines, generally putrescine and spermidine are more abundant while spermine is present in lower or trace amounts. It has to be pointed out that, due to an insufficient separation from other compounds by thin layer chromatography, some of the older data over-estimated sper-

mine content. This problem was solved by separating most of the known polyamines by HPLC methodology (Torrigiani et al., 1995).

Polyamine biosynthesis

Polyamines derive from aminoacids through decarboxylation. The main part of the carbon skeleton is given by the basic aminoacids ornithine, arginine and lysine, while methionine contributes with the aminopropyl moiety to the formation of spermidine and spermine (Fig. 1). Putrescine is normally synthesised from two major pathways: directly via ornithine decarboxylase (ODC, EC 4.1.1.17), or indirectly via arginine decarboxylase (ADC, EC 4.1.1.19). In this second reaction two important intermediates are involved: agmatine, transformed by agmatine iminohydrolase enzyme (EC 3.5.3.12) first to N-carbamoylputrescine and then to putrescine by N-carbamoylputrescine amidohydrolase enzyme (EC 3.5.1.53).

In animals and some human- and phyto-pathogenic fungi the decarboxylation of ornithine is the only possible way for putrescine synthesis, while in bacteria, other fungi and higher plants both ornithine and arginine pathways are involved. Smith and Marshall (1988) showed however that, in the presence of pyridoxal phosphate, extracts of wheat, barley, sugar beet and rape leaves were able to catalyse the release of CO_2 from the carboxyl group of ornithine, without leading to the formation of putrescine, even if no information were recently published about this pathway. This oxygen-dependent reaction is here reported:



The real ODC activity should be established by relating CO_2 release and putrescine formation taking also into consideration ornithine transcarbamoylase activity (EC 2.1.3.3), enzyme very active in higher plants, which converts ornithine back to arginine through ornithine cycle. This reaction involves citrulline and argininosuccinate (ARS) as intermediates and the action of other two enzymes ARS synthase (EC 6.3.4.5) and ARS lyase (EC 4.3.2.1) (Fig. 1). For a correct determination of ADC activity is instead necessary to consider arginase activity (EC 3.5.3.1) (Fig. 1), which catalyses the conversion of arginine to ornithine causing an over-evaluation of ADC activity, if not inhibited during the assay.

In *Sesamum* leaves and *Helianthus tuberosus* tuber explants (Crocomo and Basso, 1974; Speranza and Bagni, 1978), a citrulline decarboxylase activity, which forms N-carbamoylputrescine from citrulline, was also detected. In *Sesamum* this enzyme was responsible for putrescine synthesis only during potassium deficiency stress.

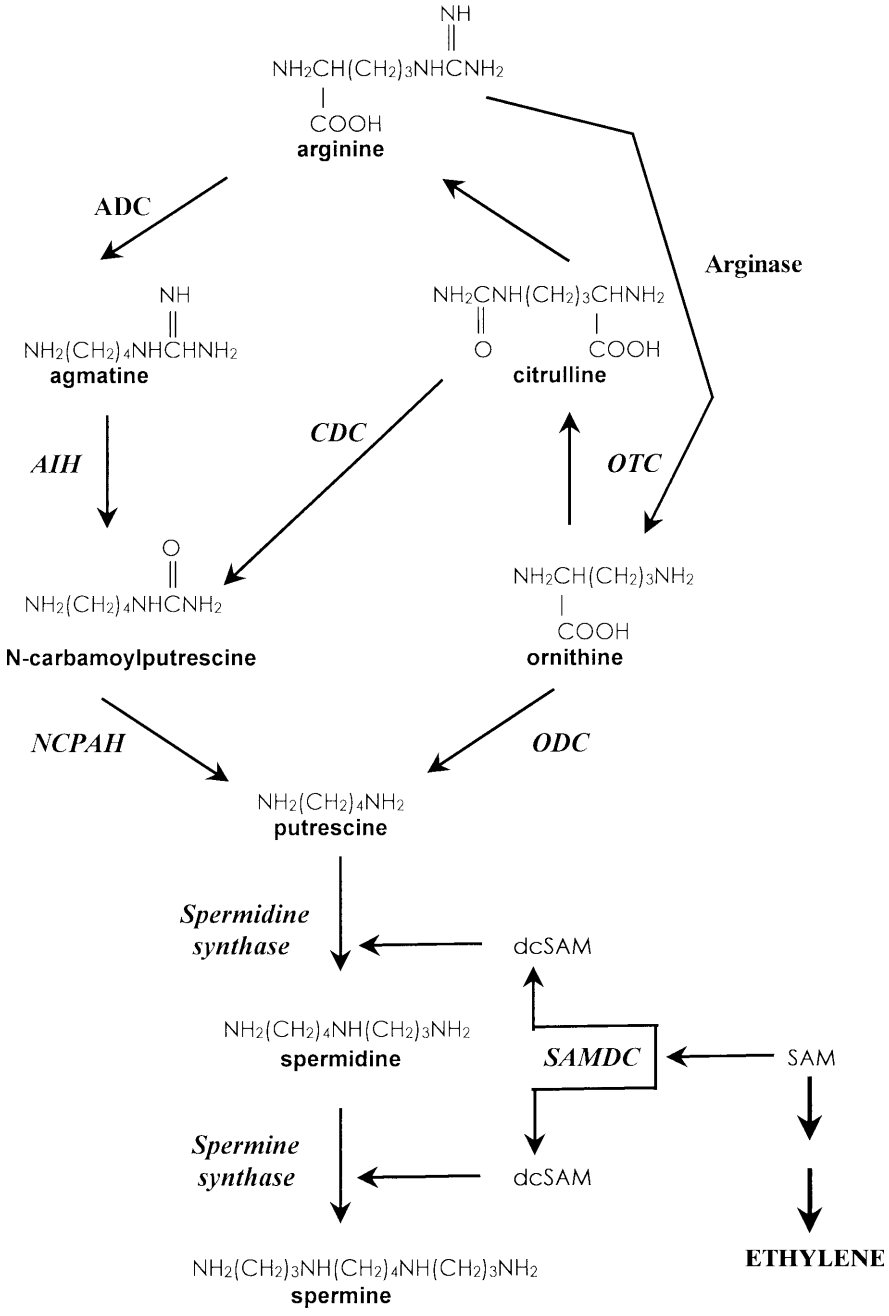


Fig. 1. Biosynthetic pathways of polyamines in plants. *ADC* arginine decarboxylase; *AIH* agmatine iminohydrolase; *CDC* citrulline decarboxylase; *dcSAM* decarboxylated S-adenosylmethionine; *NCPAH* N-carbamoylputrescine amidohydrolase; *OTC* ornithine transcarbamoylase; *SAMDC* S-adenosylmethionine decarboxylase

Cadaverine (1,5-diaminopentane) arises from lysine through lysine decarboxylase (EC 4.1.1.18). This enzyme is present in some bacteria, but also in several higher plants, in particular Gramineae, Leguminoseae and Solanaceae (Smith and Wilshire, 1975). Even if lysine represents the main cadaverine

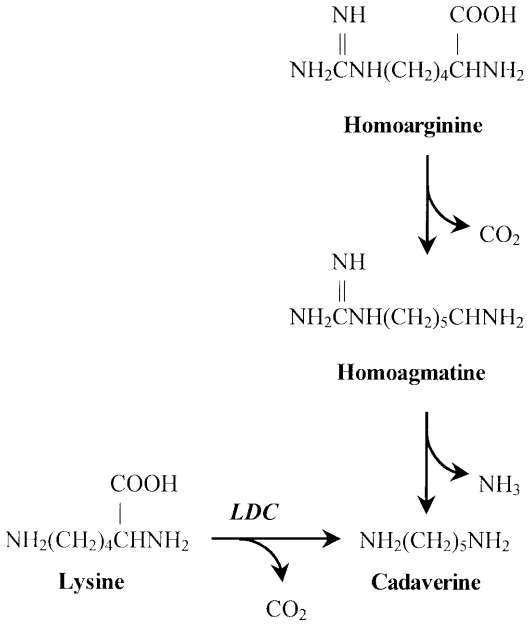


Fig. 2. Alternative pathways for cadaverine biosynthesis. *LDC* lysine decarboxylase

precursor, in some plants such as *Lathyrus sativus*, this polyamine arises from homoarginine via homoagmatine (Fig. 2) (Ramakrisna and Adiga, 1976). In Solanaceae family, cadaverine is often related to alkaloids synthesis. In *Nicotiana glauca*, for example, cadaverine is the main precursor of anabasine, whose biosynthesis takes mainly place in the roots (Bagni et al., 1986). Other polyamines, such as putrescine, can be utilised in tobacco as precursors of tropane and nicotine alkaloids (Kutchan, 1995). The distinction between alkaloids and amines is not yet clear. True amines (e.g. mescaline) have sometimes been reported as alkaloids. Alkaloids generally display the nitrogen atoms in heterocyclic groups and an higher molecular weight than amines.

No clear relationship was yet found between cadaverine and other polyamines like putrescine, spermidine and spermine. In animal tumour cells exposed to α-difluoromethylornithine (DFMO), an irreversible ODC inhibitor, cadaverine, not normally produced, was formed by a compensatory mechanism consequent to the block of putrescine and spermidine synthesis, and rapidly converted to aminopropylcadaverine and bis(aminopropyl) cadaverine, spermidine and spermine analogues (Jänne et al., 1981). Some Leguminoseae plants contain an abnormal amount of cadaverine (Federico and Angelini, 1988), which could be transformed to aminopropylcadaverine and bis(aminopropyl)-cadaverine, as also suggested by Bagni et al. (1981) in *Helianthus tuberosus* explants.

The triamine spermidine derives from the condensation, catalysed by spermidine synthase enzyme (putrescine aminopropyltransferase, PAPT, EC 2.5.1.16), of putrescine with decarboxylated S-adenosylmethionine (dcSAM, Fig. 1) previously synthesised by S-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50). The tetramine spermine arises from the further con-

densation of spermidine with dcSAM, catalysed by spermine synthase (spermidine aminopropyltransferase, SAPT, EC 2.5.1.22). In mammalian cells two distinct enzymes, spermidine (PAPT) and spermine (SAPT) synthase, substrate- and product-specific (Pegg, 1983), were found. On the contrary, bacteria have either a single enzyme (PAPT) that synthesises uniquely spermidine (Pegg, 1983), or one aminopropyltransferase with a broad substrate specificity, which produces several polyamines (Cacciapuoti et al., 1986). Bagga et al. (1997) demonstrated in the plant alfalfa *Medicago sativa* the presence of only one putrescine aminopropyltransferase also responsible for the synthesis of uncommon polyamines such as thermospermine, homocaldopentamine and homocaldohexamine.

S-adenosylmethionine, besides being an intermediate for spermidine and spermine synthesis, is also contributing to the formation of 1-aminocyclopropane-1-carboxylic acid, a precursor of ethylene hormone (Fig. 1). A number of physiological effects of ethylene in plants seem to be antagonised by polyamines treatment. Polyamines and ethylene could therefore differently influence plant growth and senescence by sharing S-adenosylmethione as intermediate (Galston and Kaur-Sawhney, 1987).

Cloning of the main polyamine biosynthetic enzymes in plants

During the last few years several genes encoding polyamine biosynthetic enzymes have been cloned in different plants. Arginine decarboxylase gene was cloned in oat (Bell and Malmberg, 1990), tomato (Rastogi et al., 1993), pea (Perez-Amador et al., 1995), *Arabidopsis thaliana* (Watson and Malmberg, 1996) and soybean (Nam et al., 1995). All these cloned ADC genes seem to be similar to the *Escherichia coli* one, encoding proteins containing a conserved putative substrate-binding site. ADC enzyme was also purified and characterised in several plants among which oat (Smith, 1979) and rice (Reggiani et al., 1994). Most of those earlier reports seem to indicate a cytosolic localisation of ADC enzyme; on the contrary Borrell et al. (1996) reported its specific association with thylakoid membranes of chloroplasts.

The ODC cDNA of *Datura stramonium* was cloned by Michael and coworkers (1996) who showed a similarity of this ODC with other eukaryotic ODCs but with the lack of two long 3′ and 5′ untranslated regions, present for example in mammalian cells, which could be involved in the enzyme regulation. ODC enzyme was purified from cytoplasm and nucleus of germinating barley seeds where was found tightly bound to chromatin (Kyriakidis, 1983). Other authors (Torrigiani et al., 1986) detected ODC activity in the particulated fraction, especially in plastids and mitochondria. ODC turnover is extremely rapid with an enzyme half-life that varies from about one hour to few minutes in some animals cells. ODC turnover is influenced by polyamines and dependent on the synthesis of a regulative protein, named antizyme, which is induced by an excess of polyamines. Antizyme, by binding with high affinity to ODC, inhibits its activity. ODC antizyme was characterised and cloned in several animal systems (Murakami et al., 1994) and evidences of its existence were found also in plants (Koromilas and Kyriakidis, 1988) where a

gene encoding for a protein having putative antizyme activity, was isolated (Michael, personal communication 1999).

S-adenosylmethionine synthase (EC 2.5.1.6), which catalyses the biosynthesis of SAM, was cloned in *Arabidopsis thaliana* by Peleman et al. (1989) and in *Saccharomyces cerevisiae* by Thomas and Surdin-Kerjan (1987) where two genes encoding for two different isoenzymes were found. S-adenosylmethionine decarboxylase (SAMDC) genes have been cloned in potato (Mad-Arif et al., 1994), spinach (Bolle et al., 1995), periwinkle (Schröder and Schröder, 1995), carnation (Chang et al., 1996), *Tritordeum* (Dresselhaus et al., 1996). Two different genes for SAMDC, which differ for the absence of an intron in SAMDC2 leader sequence, were recently cloned from *Arabidopsis thaliana* [Franceschetti, personal communication, 1999]. Earlier reports indicated SAMDC activity localised in the cytoplasm (Torrigiani et al., 1986), even if recent findings clearly evidenced the presence of this activity in the particulated fraction of tomato (Bagni, personal communication 1999).

Spermidine synthase cDNAs were isolated from *Nicotiana tabacum, Hyoscyamus niger, Arabidopsis thaliana* (Hashimoto et al., 1998) and pea fruits (Alabadí and Carbonell, 1999).

Recessive mutations in the *Arabidopsis* ACAULIS5 gene, required for internodal growth and also for the maintenance of the proliferative activity of flower-producing meristems, were identified in *Arabidopsis thaliana* (Hanzawa et al., 1997). Recent findings suggest that the product of this gene could be a spermine synthase [Hanzawa, personal communication, 1999].

Finally, the enzyme homospermidine synthase (EC 2.5.1.44), which catalyses the conversion of putrescine to homospermidine, was recently cloned in *Senecio vulgaris* (Kaiser, 1999).

Polyamines oxidation

Acetylated polyamines were discovered for the first time in urine and were related to animal excretion (Seiler et al., 1983). More recently, however, it was established that for vertebrates and yeasts, N^1-acetylation is the first step in the degradative transformation of one polyamine into another (Fig. 3) (Gillyon et al., 1987). This interconversion could regulate intracellular polyamine metabolism and was hypothesised in different plant materials, like maize roots (De Agazio et al., 1995). This process involves the action of several different enzymes (Fig. 3), among which a polyamine oxidase (EC 1.5.3.11). In plants acetylpolyamines have been detected in sugar beet seedlings (Christ et al., 1989), *Helianthus tuberosus* chloroplasts (Del Duca et al., 1995) and in different organs of *Arabidopsis thaliana* (Tassoni et al., 2000).

In addition to the breakdown of spermidine and putrescine through acetylation process, polyamines are catabolized by diamine oxidases (DAO, EC 1.4.3.6) and other polyamine oxidases (PAO, EC 1.4.3.3) specific for plants (Fig. 4). Diamine oxidases, firstly found in Leguminosae and present practically in all the monocots and dicots families tested, have a broad specificity oxidising putrescine and other diamines, but also spermidine

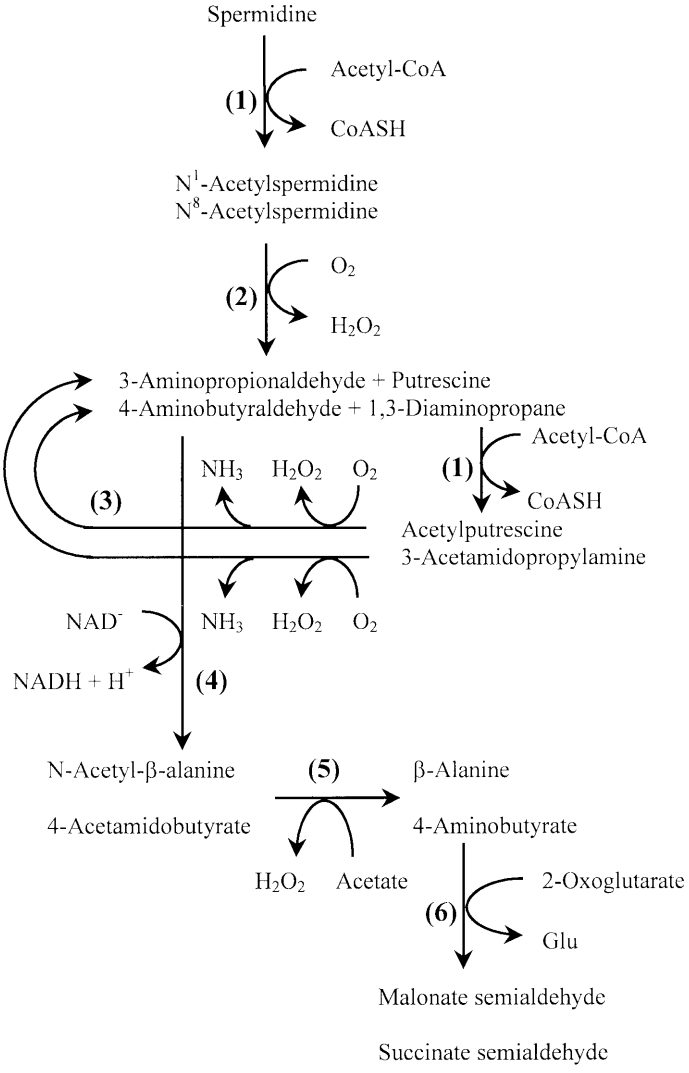


Fig. 3. Spermidine and putrescine breakdown through acetylation mechanism. This scheme represents the real pathway in animals, yeast and one the hypothesised routs in plants. Enzyme involved: (*1*) diamine acetyltransferase; (*2*) polyamine oxidase; (*3*) acetylputrescine oxidase; (*4*) acetamidoaldehyde dehydrogenase; (*5*) acetamidoalkanoate deacetylase; (*6*) ω-amino acid aminotransferase

(Fig. 4). DAO was purified at the homogeneity from clover (*Trifolium subterraneum*) leaves and three isoenzymes were identified (Delhaize and Webb, 1987). This enzyme was also purified from several plant among which soybean, *Euphorbia characias*, *Pisum sativum, Vicia faba*, *Nicotiana rustica* and rice (Smith and Barker, 1988). DAO was localised in Leguminosae apoplast (Smith and Barker, 1988), however in *Helianthus tuberosus* tuber and barley primary leaves, DAO activity appears to be symplastic, in particular compartmented in mitochondria (Scoccianti et al., 1991). A DAO obtained from pea seedlings was crystallised by Kumar and co-workers (1996). Plant polyamine oxidases, which apparently occur mainly in the cell wall of monocots, have been purified and partially characterised from few species,

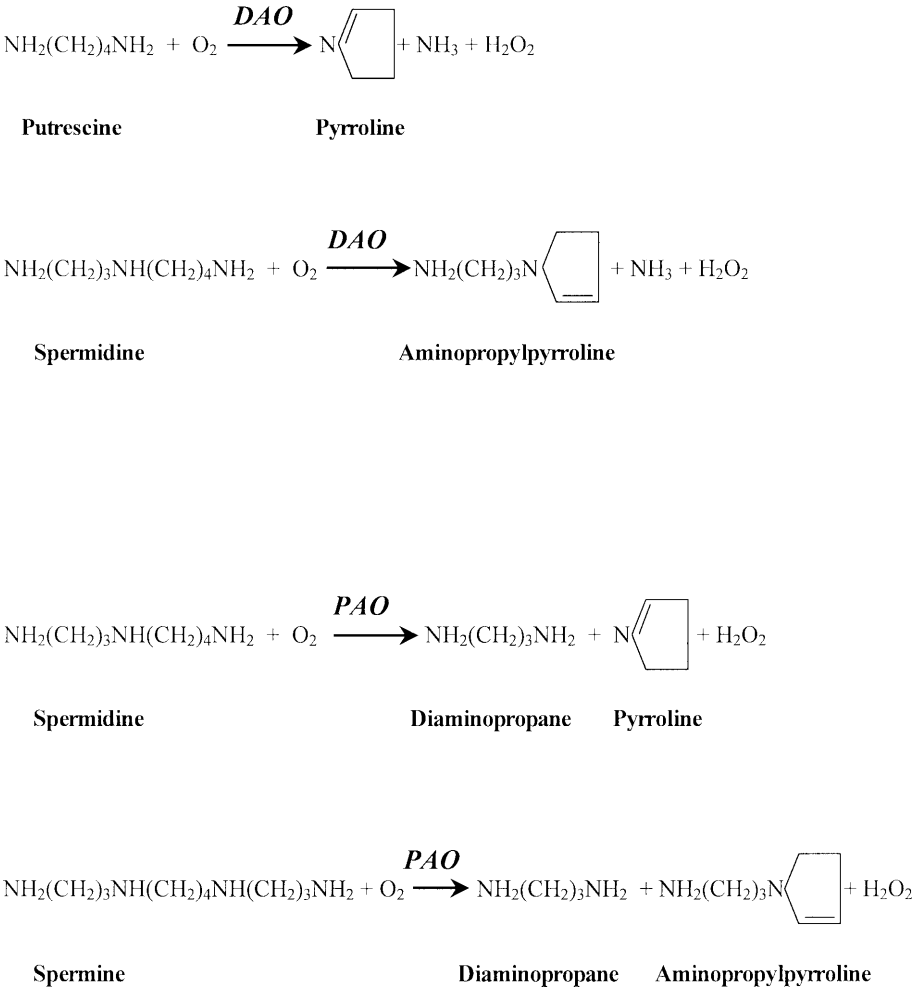


Fig. 4. Diamino oxidase and polyamine oxidase mechanisms of action in plants

especially Gramineae (Federico and Angelini, 1991). They are quite distinct from diamine oxidase (Fig. 4) and specific for spermidine, spermine and other polyamines. The complete aminoacid sequence of maize PAO, the most studied member of this enzyme class, was recently obtained by Tavaldoraki et al. (1998).

In addition to the oxidative enzymes previously mentioned, in plants is also present an heterogeneous amine oxidase (EC 1.4.3.4) which is widely distributed and has a broad range of action on primary amines, but degrades also secondary and tertiary amines (Federico and Angelini, 1991).

Another different polyamine oxidation pathway was hypothesised for a line of tobacco cells, selected for the ability to utilise putrescine as unique nitrogen source, which showed the absence of both diamine and polyamine oxidase (Balint et al., 1989). The proposed putrescine mechanism of oxidation is the following: putrescine, hydroxycinnamoylputrescine,

hydroxycinnamoyl-4-aminobutyraldehyde, hydroxycinnamoyl-4-amino-n-butyric acid (hydroxycinnamoyl-GABA), GABA. This pathway is similar to that found in animals and fungi. GABA was also found in *Pinus radiata* cotyledons fed with putrescine (Kumar and Thorpe, 1989).

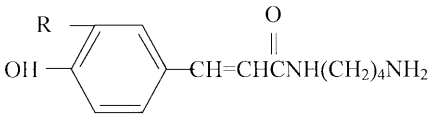
Bacteria and fungi have different polyamines degradative enzymes. Many fungi catabolize polyamines through acetyltransferase (Fig. 3), furthermore the oxidation of spermidine produces 3-aminopropanalaldehyde.

Polyamines conjugation

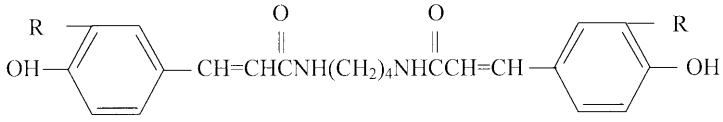
Plant polyamines may occur as free molecules but also as conjugates to small molecules, such as amides of hydroxycinnamic acids, or to proteins. Di- and polyamines conjugated with cinnamic acids were found in many plant families but in particular in Solanaceae (Smith et al., 1983). Putrescine mainly forms monomers (PCA-soluble fraction) with coumaric acid, caffeoyl acid or ferulyc acid, but can also conjugate dimers of these hydroxycinnamic acids (PCA-insoluble fraction) (Fig. 5). More recently, in the PCA-insoluble fraction of *Quercus dentata* pollen (Bokern et al., 1995), a trisubstituted hydroxycinnamic acid spermidine, was found (Fig. 5). These conjugates are of particular importance both for the regulation of polyamine concentration inside the cell (Bagni and Pistocchi, 1990), and for their interaction with cell wall components. In fact hydroxycinnamic acid bridges, through ester-ether linkages, different cell wall polymers, essentially hemicelluloses and lignin (Markwalder and Neukom, 1976; Lam et al., 1992).

The relative proportions of free and conjugated polyamines may vary among different plant species. For example in tobacco up to 90% of the polyamine pool is in the conjugated form (Torrigiani et al., 1987). In this plant the enzyme putrescine hydroxycinnamoyl transferase (EC 2.3.1.138), involved in the conjugation process, was characterised and purified (Negrel, 1989). This enzyme utilises preferably as substrates diaminopropane, putrescine, cadaverine, agmatine and spermidine, while caffeoyl- feruloyl-cinnamoyl-CoA seem to be the best donors. Notwithstanding several studies performed, the enzyme for the hydrolysis of these conjugates was not yet identified. In some cases the decrease of conjugated polyamines could be due, in the absence of a direct mechanism of hydrolysis, to the activation of hydroxycinnamic-GABA pathway (see Polyamine oxidation), hypothesis which has still to be fully demonstrated.

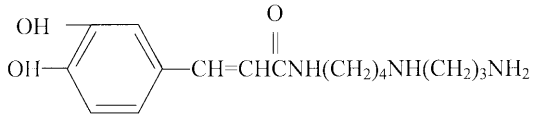
The function of conjugated polyamines is still unclear even if a positive correlation between the accumulation of these compounds and floral induction and/or bud formation was found. Many experiments performed with the aim to demonstrate the direct involvement of these compounds in floral morphogenesis, didn't give any clear result. It was in fact clarified that only polyamines in the free form are translocated (Antognoni et al., 1998) and that conjugated polyamines have no effect on cell division process as well as on floral bud primordia (Bagni et al., 1994). A role of hydroxycinnamic acid amide conjugates, but not of free polyamines, in defence mechanisms against



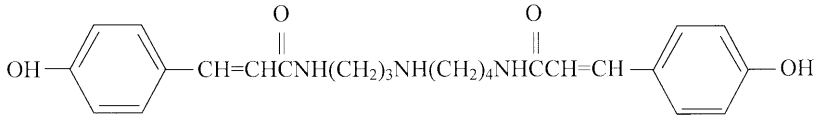
R = H **Coumaroylputrescine**
R = OH **Caffeoylputrescine**
R = OCH_3 **Feruloylputrescine**



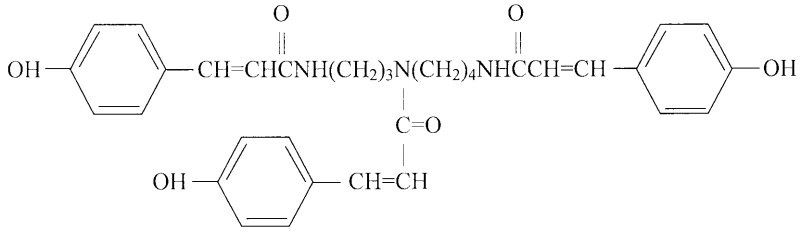
R = H **di Coumaroylputrescine**
R = OCH_3 **di Feruloylputrescine**



Caffeoylspermidine



di Coumaroylspermidine



Tricoumaroylspermidine

Fig. 5. Conjugates of hydroxycinnamic acids and polyamines

biotic and abiotic stress, mainly acting as radical scavengers, was also proposed (Bors et al., 1989).

Other polyamine conjugates are represented by hordatines which are dimers of coumaroylagmatine found in barley seedlings and have antifungal properties (Smith et al., 1983).

A transglutaminase enzyme, capable of catalysing the covalent binding of polyamines to proteins, was also found in plants. This enzyme seems to be widespread and involved in important biological functions such as in the organisation of cytoskeletal proteins in pollen tube elongation (Del Duca et al., 1997) and photosynthesis. In particular, the polyamine conjugation, due to transglutaminase, to thylakoid and stromatal proteins of antenna complexes, especially to ribulose bisphosphate carboxylase/oxigenase (Rubisco), seems to have an important role in protecting these proteins from protease action, preserving photosynthetic efficiency (Serafini-Fracassini et al., 1995).

Concluding remarks

All the studies performed with the aim of directly establish polyamine compartmentation were partly limited by experimental difficulties and polyamine redistribution during tissue extraction. It seemed therefore easier to study compartmentation through the localisation of the enzyme responsible for polyamine biosynthesis or oxidation. At the present time SAMDC seems to be preferentially located in the cytosolic fraction, while ODC and ADC seem to be mainly particulated, distributed in nuclei, chloroplasts and mitochondria.

The function and significance of putrescine biosynthetic main enzymes (ADC and ODC), is still a matter of debate.

A simple hypothesis is that ADC and ODC, by having a separate localisation within cellular compartments, have different functions. It was suggested that ODC is mainly involved in cell division, while ADC is related to stress phenomena (biotic and abiotic) and/or to cell extension (Bagni, 1989), even if no conclusive data were obtained. Moreover the existence of different isoenzymes at least for SAMDC, but not, up to now, for ODC, ADC and spermidine synthase, is in favour of specific and localised functions inside the plant cell. Additional informations derive from the studies on compartmentation and metabolic regulation of the enzymes involved in ornithine and arginine biosynthesis. In fact, arginine conversion to ornithine via arginase, occurring in mitochondria appears to be distinct from arginine synthesis located partly in the cytoplasm (Shargool et al., 1988). Mitochondria and chloroplast membranes should therefore represent a barrier which separates ornithine as a catabolic product from ornithine destined to arginine biosynthesis.

Several events seem though to participate to the regulation of polyamine cytoplasmatic levels: the presence of polyamines stored in the vacuole and in other compartments, such as mithocodria and chloroplasts, the polyamine interaction with cell wall constituents, the existence of conjugated polyamines and of different oxidases.

Further studies on immunolocalization of all the enzymes involved in polyamine metabolism and a complete study of their expression at intracellular level are in progress, and will be of great importance in understanding the role of polyamines in plant cells.

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