Plant Physiol. (1997) 114: 445–454

Putrescine Aminopropyltransferase Is Responsible for Biosynthesis of Spermidine, Spermine, and Multiple Uncommon Polyamines in Osmotic Stress-Tolerant Alfalfa[1]

Suman Bagga, James Rochford, Zita Klaene, Glenn D. Kuehn, and Gregory C. Phillips*

Department of Agronomy and Horticulture (S.B., J.R., Z.K., G.C.P.), and Department of Chemistry and Biochemistry (G.D.K.), Molecular Biology Program, New Mexico State University, Las Cruces, New Mexico 88003

The biosynthesis of polyamines from the diamine putrescine is not fully understood in higher plants. A putrescine aminopropyltransferase (PAPT) enzyme activity was characterized in alfalfa (*Medicago sativa* L.). This enzyme activity was highly specific for putrescine as the initial substrate and did not recognize another common diamine, 1,3-diaminopropane, or higher-molecular-weight polyamines such as spermidine and spermine as alternative initial substrates. The enzyme activity was inhibited by a general inhibitor of aminopropyltransferases, 5′-methylthioadenosine, and by a specific inhibitor of PAPTs, cyclohexylammonium sulfate. The initial substrate specificity and inhibition characteristics of the enzyme activity suggested that it is a classical example of a PAPT. However, this enzyme activity yielded multiple polyamine products, which is uncharacteristic of PAPTs. The major reaction product of PAPT activity in alfalfa was spermidine. The next most abundant products of the enzyme reaction using putrescine as the initial substrate included the tetramines spermine and thermospermine. These two tetramines were distinguished by thin-layer chromatography to be distinct reaction products exhibiting differential rates of formation. In addition, the uncommon polyamines homocaldopentamine and homocaldohexamine were tentatively identified as minor enzymatic reaction products but only in extracts prepared from osmotic stress-tolerant alfalfa cultivars. PAPT activity from alfalfa was highest in meristematic shoot tip and floral bud tissues and was not detected in older, nonmeristematic tissues. Product inhibition of the enzyme activity was observed after spermidine was added into the in vitro assay for alfalfa PAPT activity. A biosynthetic pathway is proposed that accounts for the characteristics of this PAPT activity and accommodates a novel scheme by which certain uncommon polyamines are produced in plants.

The diamine putrescine and the polyamines spermidine and spermine are ubiquitous components of living organisms and are essential primary metabolites for normal growth and development (Flores et al., 1989). These three compounds are often referred to as common polyamines, and polyamines with a restricted natural occurrence may be referred to as uncommon polyamines (Phillips and Kuehn, 1991). Although the biosynthesis of putrescine is relatively well understood, little is known or directly confirmed in plants regarding the enzymes that catalyze the synthesis of spermidine and spermine from putrescine (Suzuki et al., 1990; Slocum, 1991).

Based on the paradigm in mammalian systems (Hibasami et al., 1980a; Pegg, 1983), it is generally assumed that spermidine synthase (PAPT, EC 2.5.1.16) and spermine synthase (SAPT, EC 2.5.1.22) are two separate enzymes in plants, both requiring decarboxylated *S*-adenosylmethionine as the propylamine donor (Flores et al., 1989; Slocum, 1991). Apparently, two specific enzymes are required because the two consecutive propylamine transfers are made to opposing ends of the original putrescine molecule. However, PAPT has been partially purified from only two higher plant sources (Hirasawa and Suzuki, 1983; Yamanoha and Cohen, 1985) and appears to be a soluble cytoplasmic enzyme (Sindhu and Cohen, 1984a). SAPT has not been characterized in any plant, although detection of its activity has been reported in a rare case (Sindhu and Cohen, 1984b).

Mammalian PAPTs and SAPTs have high specificity for their substrates (putrescine and spermidine, respectively) and products (spermidine and spermine, respectively) (Hibasami et al., 1980a; Pegg, 1983). Most bacteria have only the PAPT, with a similar high specificity for putrescine as the

[1] This research was supported in part by grants from the U.S. Department of Agriculture (USDA), the Southwest Consortium on Plant Genetics and Water Resources (grant no. 92-34186-7249), the USDA National Research Initiative (grant no. 93-37301-9429), and the New Mexico Agricultural Experiment Station.

* Corresponding author; e-mail grphilli@nmsu.edu; fax 1–505–646–6041.

Abbreviations: caldopentamine, 1,15-diamino-4,8,12-triazapentadecane [$H_2N(CH_2)_3NH_2(CH_2)_3NH_2(CH_2)_3NH_2(CH_2)_3NH_2$]; CHA, cyclohexylammonium sulfate; DAP, 1,3-diaminopropane [$H_2N(CH_2)_3H_2$]; dSAM, decarboxylated *S*-adenosylmethionine; homocaldohexamine, 1,20-diamino-4,8,12,16-tetra-azaeicosane [$H_2N(CH_2)_3NH_2(CH_2)_3NH_2(CH_2)_3NH_2(CH_2)_3NH_2(CH_2)_4NH_2$]; homocaldopentamine, 1,16-diamino-4,8,12-triazahexadecane [$H_2N(CH_2)_3NH_2(CH_2)_3NH_2(CH_2)_3NH_2(CH_2)_4NH_2$]; MTA, 5′-methylthioadenosine; norspermidine, 1,7-diamino-4-azaheptane [$H_2N(CH_2)_3NH_2(CH_2)_3NH_2$]; norspermine, 1,11-diamino-4,8-diazaundecane [$H_2N(CH_2)_3NH_2(CH_2)_3NH_2N(CH_2)_3NH_2$]; PAPT, putrescine aminopropyltransferase; putrescine, 1,4-diaminobutane [$H_2N(CH_2)_4NH_2$]; Rm, relative migration distance; SAPT, spermidine aminopropyltransferase; spermidine, 1,8-diamino-4-azaoctane [$H_2N(CH_2)_3NH_2(CH_2)_4NH_2$]; spermine, 1,12-diamino-4,9-diazadodecane [$H_2N(CH_2)_3NH_2(CH_2)_4NH_2(CH_2)_3NH_2$]; thermospermine, 1,12-diamino-4,8-diazadodecane [$H_2N(CH_2)_3NH_2\ (CH_2)_3NH_2(CH_2)_4NH_2$].

substrate and spermidine as the product and no corresponding SAPT, because spermine is not synthesized in most prokaryotes (Pegg, 1983). However, some thermophilic and acidophilic bacteria possess a single aminopropyltransferase with broad substrate specificity, permitting the biosynthesis of a variety of individual common and uncommon polyamines from the separate substrates (Cacciapuoti et al., 1986). The only known propylamine donor for polyamine synthesis, besides decarboxylated *S*-adenosylmethionine, which is utilized by aminopropyltransferases, is L-aspartyl-β-semialdehyde (Phillips and Kuehn, 1991). This propylamine donor is used by *Lathyrus sativus* L. (Srivenugopal and Adiga, 1980), by cultured tobacco cells (Lee and Park, 1991), and by a few bacteria (Tait, 1985) in an alternative route to spermidine synthesis and in homospermidine synthesis.

The objective of the research presented in this paper was to characterize the aminopropyltransferase activities in an osmotic stress-tolerant strain of alfalfa (*Medicago sativa* L.) that we have used in previous studies (Kuehn et al., 1990a). The evidence presented here suggests that PAPT, but not SAPT, activity is present in this alfalfa system. Moreover, this PAPT in alfalfa, utilizing putrescine as the sole initial substrate, yields as its products not only spermidine but also spermine, thermospermine, and, putatively, homocaldopentamine and homocaldohexamine. These latter three uncommon polyamines are of interest because of the previous implication that high-molecular-weight uncommon polyamines may be involved in mediating the growth response of various organisms under extreme environmental conditions (Kuehn et al., 1990a; Phillips and Kuehn, 1991).

MATERIALS AND METHODS

Meristematic tissue from shoot tips and immature floral buds was obtained from a plot of field-grown alfalfa (*Medicago sativa* L.) containing a mixture of plants from the cvs Mesilla, Zia, and Wilson. Vegetative and reproductive tissues from whole plants were obtained from two different strains of alfalfa, Mesilla-0 (osmotic stress-susceptible) and Mesilla-3 (osmotic stress-tolerant), which were grown in a rainout shelter, as described in our previous work (Kuehn et al., 1990a). Alfalfa seeds from Mesilla-0 and Mesilla-3 were also grown in the laboratory and the seedlings were harvested after 10 d for enzyme assays. Tissues were bulked from 10 to 15 different plants to prepare a single sample replication for analysis, and each data point presented in the tables and figures represents the mean of at least three replications.

Chemicals

dSAM was prepared in the laboratory essentially by the method of Wickner et al. (1970) using the *S*-adenosylmethionine decarboxylase activity from *Escherichia coli* strain K-12. [Tetramethylene-1,4-^{14}C]putrescine dihydrochloride (73.9 mCi mmol^{-1}), [tetramethylene-1,4-^{14}C]-spermidine trihydrochloride (73.2 mCi mmol^{-1}), and [tetramethylene-1,4-^{14}C]spermine tetrahydrochloride (73.2 mCi mmol^{-1}) were purchased from Dupont-New England Nuclear. Unlabeled samples of these three polyamines were purchased from Sigma. DAP, norspermidine, and norspermine were purchased from Kodak. DAP was tritiated by Dupont-NEN by the Wilzbach method of catalytic ^{3}H exchange, purified using Dowex 50W × 400-mesh cation exchange, and recovered as 320 mCi mmol^{-1}. Samples of synthetic caldopentamine and homocaldopentamine (Niitsu and Samejima, 1986) used as standards were gifts of Dr. K. Hamana (Gunma University, Japan). Other reagents were of an analytical grade or better.

Preparation of Enzyme Extract

Approximately 5 g fresh weight of alfalfa tissue was frozen in liquid N_2 and ground to a fine powder with a pestle in a prechilled mortar for each sample. The powder was homogenized (Omni mixer, Sorvall) in 5 volumes of ice-cold 0.1 M potassium phosphate buffer, pH 7.4, at full power for 2 to 3 min. All subsequent operations were performed at 0 to 4°C. The homogenate was filtered through four layers of cheesecloth and centrifuged at 100,000*g* for 60 min. The resulting supernatant fraction of approximately 4 mL was applied to a Sephadex G-50 column (1.5 × 15.0 cm), equilibrated with 0.1 M potassium phosphate buffer, and eluted with the same buffer to collect 1-mL fractions. The protein concentrations of the individual fractions were determined by colorimetric assay using BSA as a standard (Bradford, 1976). The active fractions were pooled and stored at −20°C in 1-mL samples.

In Vitro Aminopropyltransferase Enzyme Reactions

The reaction conditions to test for aminopropyltransferase activities were modified from those of Tabor and Tabor (1983). The assay mixture contained (in a 100-μL final volume) 150 mM potassium phosphate buffer, pH 7.4, 5 mM DTT, 2 mM dSAM, 1 × 10^{5} dpm of [^{14}C]putrescine, [^{14}C]spermidine, [^{14}C]spermine, or [^{3}H]diaminopropane (specific radioactivities indicated above) as alternative initial substrates, and 100 μg of protein extract. The reaction was incubated at 37°C for varying times and was terminated by the addition of 10 μL of 60% TCA. In routine assays, the reaction time was terminated after either 30 min (when only the primary reaction products, spermidine and spermine/thermospermine, were quantitated) or 60 min (when both the primary reaction products and the minor reaction products, putative homocaldopentamine and homocaldohexamine, were quantitated). The denatured protein was then removed by centrifugation at 12,000*g*, and the supernatant fraction was used for separation and quantitation of the enzymatic reaction products.

Paper Electrophoresis

The separation of radiolabeled polyamine products formed by in vitro enzyme reactions was performed by paper electrophoresis, using a method modified from the procedures of Tabor and Tabor (1983). A sample solution (11 μL) of the centrifuged reaction mixture and 0.5 μmol each of unlabeled carriers were streaked across the short dimension of a 4- × 15-cm paper strip (3MM chromatog-

raphy paper, Whatman) saturated in 44 mM sodium citrate, pH 4.3. Electrophoresis was carried out at 200 V and 12 mA using an electrophoresis chamber (model 38201, Gelman Instruments, Ann Arbor, MI) for 1 h in the same citrate buffer at room temperature. After electrophoresis the paper strips were air-dried at room temperature and then heated to 80°C for 5 to 10 min in an oven. Strips prepared with standards were sprayed with ninhydrin reagent (0.2% ninhydrin in 1-butanol saturated with water). The ninhydrin-sprayed paper strips were developed at 80°C for 5 to 10 min in an oven. The separated zones of polyamine products were identified by comparison with the sprayed standards and were then cut from the strip and counted in 3 mL of nonaqueous scintillation cocktail (Margosiak et al., 1990) with a liquid scintillation spectrometer (model CA 2000 Tri-Carb, Hewlett-Packard).

The Rm values for different polyamines, indicated by the stained zones, were determined by measuring the distance from the point of application to the edge of the zone nearest to the cathode and dividing this measurement by the distance from the point of application that putrescine migrated. Putrescine was used as the control reference (Rm = 1.00). Typical Rm values were: putrescine, 1.00; spermidine, 0.70; spermine, 0.68; DAP, 1.00; norspermidine, 0.80; and norspermine, 0.67. The specific enzyme activities were calculated as units per milligram of protein. One unit of enzyme activity represents the formation of 1 pmol of spermidine (or other polyamine) product formed in 1 min under standard reaction conditions.

TLC

TLC was performed to separate several polyamine products formed during the aminopropyltransferase enzyme assays. The TLC system used was that of Shirahata et al. (1983), which uses precoated silica gel plates (X Silica Gel 60F-254E, Merck, Darmstadt, Germany) with a developing solvent system of *n*-butanol:acetic acid:pyridine:formaldehyde (3:3:2:1, v/v).

Supernatant solutions containing the aminopropyltransferase reaction products as described above were chromatographed on a 1- × 7-cm column of Dowex 50W × 8 (400-mesh H^+ form) cation-exchange resin (Fujita et al., 1980) equilibrated with 6 N HCl. The column with the applied sample was washed with at least 3 column volumes of 1 N HCl and the polyamines were eluted with at least 3 column volumes of 6 N HCl. One-milliliter fractions were collected. A sample of 10 μL from each fraction was counted in a liquid scintillation counter to determine the radioactivity. The fractions containing radioactivity were pooled and dried completely in a speed-vacuum apparatus and then redissolved in 5 μL of distilled water. This sample was applied to the TLC plate, along with standards, and chromatographed until the solvent front crossed three-fourths of the plate. TLC plates were air-dried at room temperature and baked at 80°C for 5 min. Plates were cooled and exposed to radiographic film.

HPLC of Uncommon Polyamines

HPLC separations of dansylated polyamines were performed as described by Rodriguez-Garay et al. (1989). Enzyme reaction products from alfalfa extracts, which were first separated by paper electrophoresis, were eluted from the paper strips using 6 N HCl and then prepared for HPLC analysis. Dansylated samples of synthetic caldopentamine and homocaldopentamine were used as standards for HPLC separations, in addition to commercially available standards of diamines, triamines, and tetramines.

RESULTS

Substrate Specificity of Alfalfa Aminopropyltransferase

Several polyamines were tested as the initial substrates for alfalfa aminopropyltransferase enzyme activities in a number of independent experiments. Under the reaction assay conditions used, about 80% of the radiolabel was recovered as polyamine products using [^{14}C]putrescine as the initial substrate (Table I). Multiple polyamine products were detected when putrescine was used as the initial substrate. As expected, the primary product from this reaction was spermidine. The second most abundant product(s) of this reaction migrated to the position of the tetramine standards. We subsequently identified both spermine and thermospermine as tetramine products of the reaction,

Table I. *Polyamine products of alfalfa aminopropyltransferase activity using different initial substrates*

Enzyme reactions were carried out for 60 min using partially purified protein extracts prepared from immature reproductive tissues collected from field-grown plants in a mixed plot comprised of cvs Mesilla, Zia, and Wilson. Reaction products were separated by paper electrophoresis, and radiolabeled conversions from each substrate into the respective reaction products were quantified by liquid scintillation spectrometry (mean values of three replications each, comprising samples from at least 10 different plants).

^{14}C-Labeled Initial Substrate	Products of Enzyme Reaction			
	Spermidine	Spermine and Thermospermine	Homocaldopentamine	Homocaldohexamine
	pmol min^{-1} mg^{-1} protein			
Putrescine	46.09	5.38	4.65	10.32
Spermidine	–	Not detected	1.56	2.78
Spermine	–	–	0.03	Not detected

which comigrate during paper electrophoresis but are separable by TLC (see below for details). Two high-molecular-weight polyamines were also produced by the reaction and were tentatively identified as the uncommon polyamines homocaldopentamine and homocaldohexamine (see below for details). Boiled alfalfa protein extracts used as controls produced no reaction products, and the reaction was absolutely dependent on the presence of dSAM as a propylamine donor (data not shown).

No significant aminopropyltransferase activity was detected when [^{14}C]spermidine or [^{14}C]spermine were used as the initial substrates (Table I). There was no detection of the expected product (spermine) in the reaction using spermidine as the initial substrate. This reaction did result in trace amounts of the two high-molecular-weight uncommon polyamines tentatively identified as homocaldopentamine and homocaldohexamine being formed as products; however, these amounts were only marginally above background. In addition, no polyamine products were recovered when [^{3}H]DAP was used as the initial substrate (data not shown). Thus, the alfalfa aminopropyltransferase was specific only for putrescine as the initial substrate and, therefore, it can properly be called a PAPT.

Time Course of Alfalfa PAPT Activity

Two independent studies were conducted to monitor the progress of alfalfa PAPT product formation over time. With respect to the major product formed, the time-course studies showed rapid formation of spermidine during the first 5 min and a linear rate of spermidine formation between 5 and 20 min (Fig. 1). Spermidine formation appeared to level off between 20 and 45 min of the reaction time and decline by 60 min. The rate of tetramine formation (spermine and thermospermine, which comigrate upon electrophoresis) was slower than for spermidine. The rate of tetramine formation was linear up to 15 min after initiation of the reaction. Tetramine formation appeared to remain level between 15 and 45 min, with a slight decline by 60 min. The rates of putative homocaldopentamine and homocaldohexamine formation (data not shown) were much slower than for the major reaction products, with maximum accumulation appearing between 45 and 60 min. Although the formation of spermidine (and possibly the tetramines) began to decline by 60 min, most of the subsequent enzyme assays were routinely carried out for 60 min to detect and quantify the minor, high-molecular-weight polyamine reaction products. Some enzyme assays were terminated after 30 min, when only the primary reaction products were quantitated.

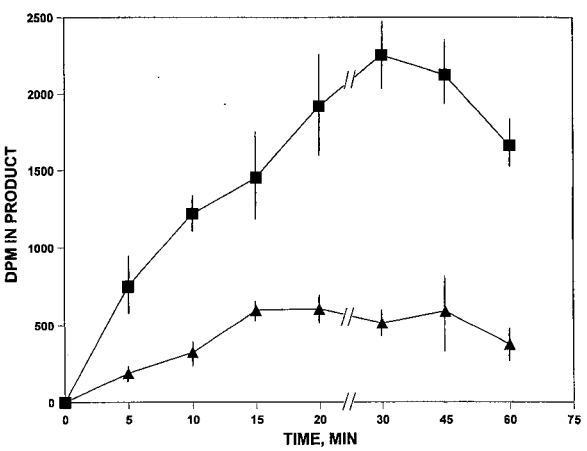

Figure 1. Time course of the formation of the major polyamine products (■, spermidine; ▲, spermine/thermospermine) by PAPT activity derived from alfalfa. Enzyme reactions were carried out using partially purified protein extracts prepared from immature reproductive tissues collected from field-grown plants in a mixed plot comprised of the alfalfa cvs Mesilla, Zia, and Wilson. Reaction products were separated by paper electrophoresis, and radiolabeled conversions from [^{14}C]putrescine substrate into the reaction products were quantified by liquid scintillation spectrometry (means ± SD of three replications, each comprising samples from at least 10 different plants).

Inhibition of PAPT Activity by Classical Inhibitors

PAPT activity in alfalfa was inhibited by the specific inhibitor of mammalian and bacterial PAPT enzymes (Hibasami et al., 1980b; Flores et al., 1989), CHA, and by the general inhibitor of aminopropyltransferase enzymes (Hibasami et al., 1980a), MTA. Approximately 90% inhibition of spermidine formation was observed at 0.5 mM CHA and above (Fig. 2A), and the K_i for spermidine formation was 0.38 mM CHA. The K_i for tetramine (spermine and thermospermine) formation was 0.40 mM CHA, for putative homocaldopentamine formation it was 0.46 mM CHA, and for putative homocaldohexamine formation it was 0.28 mM CHA. Approximately 50% inhibition of spermidine formation was observed at 1.35 mM MTA (Fig. 2B). Inhibition of the formation of the other reaction products closely paralleled the patterns seen for the major reaction product, spermidine (Fig. 2).

PAPT Activity in Different Alfalfa Cultivars and Tissues

PAPT activity was determined in meristematic tissues (shoot buds and immature floral buds) of two different cultivars of alfalfa. The cv Mesilla-0 exhibits water-use inefficiency and drought susceptibility. Mesilla-3 was selected for its increased tolerance to drought and because it exhibits enhanced water-use efficiency under field conditions. Enzyme activity was determined on water-deficit-stressed samples from mature plants of the two cultivars (Kuehn et al., 1990a). Spermidine and tetramines were formed by Mesilla-0 in the PAPT reaction, but no high-molecular-weight polyamines (putative homocaldopentamine and homocaldohexamine) were recovered as products (Table II). In contrast, Mesilla-3 formed the high-molecular-weight polyamines, as well as spermidine and tetramines, as products of the PAPT reaction.

Extracts from various types of alfalfa plant tissues were tested for PAPT enzymatic activity. Very little activity was detectable in roots, stem internodes, or fully expanded leaves (data not shown). However, PAPT activity was detected in the seedling tissues of Mesilla-0 and Mesilla-3 (Table II). There was an appreciable difference in the pro-

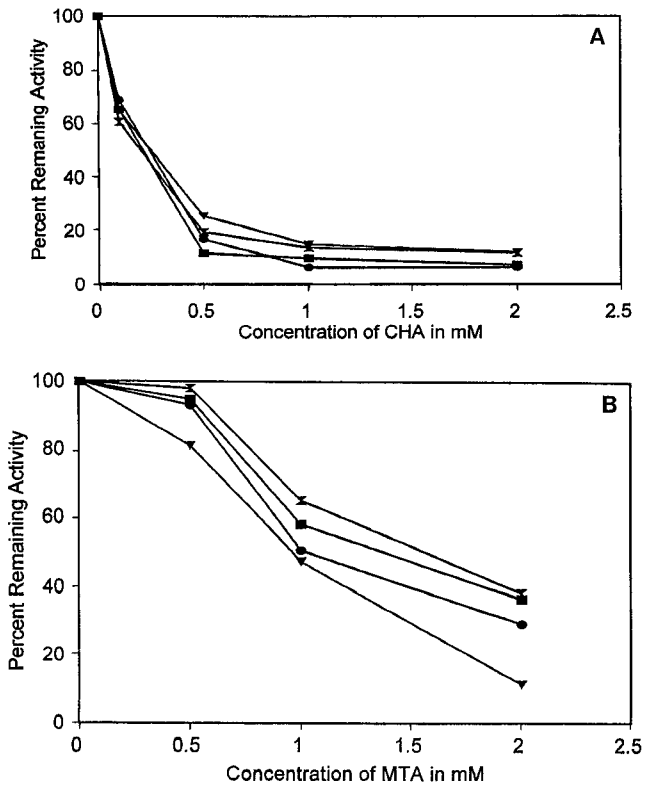

Figure 2. Inhibition of alfalfa PAPT activity by the specific inhibitor of PAPTs, CHA (A), and by the general inhibitor of aminopropyltransferases, MTA (B). Enzyme reactions were carried out for 60 min using partially purified protein extracts prepared from immature reproductive tissues collected from field-grown plants in a mixed plot comprised of the alfalfa cvs Mesilla, Zia, and Wilson. Reaction products (■, spermidine; ▼, spermine/thermospermine; ●, putative homocaldopentamine; and ⧗, putative homocaldohexamine) were separated by paper electrophoresis, and radiolabeled conversions from the [^{14}C]putrescine substrate into the reaction products were quantified by liquid scintillation spectrometry (mean values of three replications, each comprising samples from at least 10 different plants).

file of polyamine products in seedling tissues compared with mature plant meristematic tissues (Table II).

Distinction between Spermine and Thermospermine as Enzyme Reaction Products

The tetramine products of the alfalfa PAPT activity, spermine and thermospermine, cannot be separated by ion-exchange chromatography, reverse-phase HPLC, or paper electrophoresis. Spermine and thermospermine comigrate during such procedures because they are structural isomers with an identical chemical composition. A standard for thermospermine is not commercially available. A TLC method with a solvent system containing formaldehyde (Shirahata et al., 1983) was suitable for separation of spermine and thermospermine from among the products formed by alfalfa PAPT enzyme assays. Because the substrate used in the assays was radiolabeled, the reaction products were visualized as distinct spots on a developed x-ray film incubated with the TLC plates (Fig. 3). Three major spots were separable from the 1-min PAPT assay, whereas five spots were visualized from the 60-min reaction. The three spots from the 1-min reaction and three of the five spots from the 60-min reaction (R_F values of 0.62, 0.58, and 0.34) corresponded to the putrescine (the initial substrate visualized in the 0-min reaction), spermidine, and spermine standards, respectively (Fig. 3). However, the other two spots from the 60-min reaction (R_F values of 0.46 and 0.16) were unique and were not formed by the 1-min reaction. The spot at R_F 0.46 was matched to the R_F value of thermospermine, as documented in the literature (Shirahata et al., 1983). The unique spot from the 60-min reaction at R_F 0.16 could not be correlated with any of the available standards, but we later tentatively identified it as homocaldopentamine (see below for details).

The migration behaviors of spermidine, thermospermine, and spermine in our experiments were consistently slightly faster than the previously published R_F values. The R_F values of the radioactive and nonradioactive standards were comparable to those of the reaction products. How-

Table II. *Polyamine products of PAPT activity from mature plants or seedlings of osmotic stress-tolerant (Mesilla-3) and -susceptible (Mesilla-0) alfalfa cvs*

Enzyme reactions were carried out for 60 min using partially purified protein extracts. Reaction products were separated by paper electrophoresis, and radiolabeled conversions from the [^{14}C]putrescine substrate into the reaction products were quantified by liquid scintillation spectrometry. Mature plant tissue samples were comprised of a composite of immature reproductive and meristematic shoot tip tissues collected from plants grown in a rainout shelter and water-deficit-stressed as described by Rodriguez-Garay et al. (1989) and Kuehn et al. (1990a) (mean values of three replications each, comprising samples from at least 10 different plants). Seedling tissue samples were comprised of entire seedlings germinated under aseptic conditions in the laboratory (mean values of three replications each, comprising tissues from at least 15 different seedlings).

Type of Sampled Tissue	Genetic Background	Products of Enzyme Reaction			
		Spermidine	Spermine and Thermospermine	Homocaldopentamine	Homocaldohexamine
		pmol min^{-1} mg^{-1} protein			
Mature plants	Mesilla-0	69.55	5.35	Not detected	Not detected
Mature plants	Mesilla-3	47.84	6.27	3.70	2.16
Seedlings	Mesilla-0	0.25	3.59	0.45	Not detected
Seedlings	Mesilla-3	1.81	2.51	5.65	Not detected

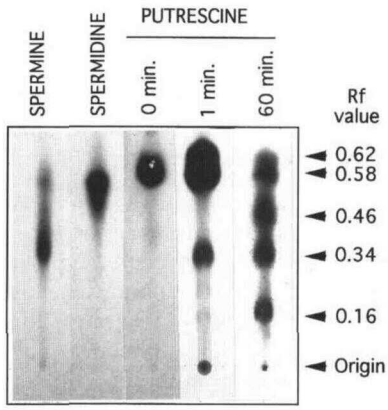

Figure 3. TLC separation of polyamine products of alfalfa PAPT activity. Enzyme reactions were carried out using partially purified protein extracts prepared from immature reproductive tissues collected from field-grown alfalfa plants in a mixed plot comprised of the cvs Mesilla, Zia, and Wilson. The reaction products formed from the [^{14}C]putrescine substrate were separated by TLC following the method of Shirahata et al. (1983). TLC plates were subjected to autoradiographic development to visualize the ^{14}C-labeled initial substrate (putrescine, in the 0-min reaction) and reaction products (1- and 60-min reactions). R_F values for the reaction products were compared with those of ^{14}C-labeled standards of spermidine and spermine and the published results of Shirahata et al. (1983). Initial substrate and reaction products were identified by R_F values, as follows: 0.62 = putrescine; 0.58 = spermidine; 0.46 = thermospermine; 0.34 = spermine; 0.16 = putative homocaldopentamine.

ever, the radiolabeled and unlabeled standards of spermine were slightly different (not shown). The nonradioactive spermine standard corresponded well with the alfalfa PAPT reaction product (R_F 0.34), whereas the radiolabeled spermine standard corresponded well with the published R_F value of 0.26 (Shirahata et al., 1983).

Tentative Identification of Other Uncommon Polyamines as Enzyme Reaction Products

Standards for the uncommon polyamines homocaldopentamine and homocaldohexamine are not commercially available and are difficult to synthesize. Small amounts of synthetic caldopentamine and homocaldopentamine were provided to us as a gift (K. Hamana, Gunma University, Japan). These samples were analyzed by HPLC and compared with alfalfa PAPT reaction products eluted from the paper electrophoresis strips. The dansylated samples of synthetic caldopentamine and homocaldopentamine exhibited HPLC retention times of 59 and 60 to 62 min, respectively (traces not shown). However, whereas the caldopentamine standard showed a characteristic single peak, the homocaldopentamine standard exhibited a cluster of three peaks. We interpreted the cluster of peaks for the homocaldopentamine standard to indicate potential contamination of the standard with by-products of the synthesis or dansylation procedures. The alfalfa PAPT reaction product suspected to be a pentamine showed a single peak at a retention time of 60 min, which overlapped with one of the peaks exhibited by the standard for homocaldopentamine. We could not conclusively identify the PAPT reaction product as homocaldopentamine, because the synthetic standard showed additional but unexpected peaks and was therefore suspected of being contaminated. Thus, we tentatively identified the PAPT reaction product as homocaldopentamine. The samples of the synthetic standards, as well as the PAPT reaction products, were insufficient in amount (and possibly in purity) for a more definitive analysis by HPLC-MS or other procedures.

In addition, the PAPT reaction product suspected to be homocaldohexamine was analyzed by HPLC and showed a characteristic single peak at a retention time of 76 min. This retention time would be expected for homocaldohexamine,

Table III. *Inhibition of alfalfa PAPT activity in vitro by the major reaction product, spermidine*

Enzyme reactions were carried out for 30 min using partially purified protein extracts prepared from immature reproductive tissues collected from field-grown plants in a mixed plot comprising cvs Mesilla, Zia, and Wilson. Reaction products were separated by paper electrophoresis, and radiolabeled conversions from [^{14}C]putrescine substrate into the reaction products were quantified by liquid scintillation spectrometry (mean values of three replications each, comprising samples from at least 10 different plants). Specific activity of spermidine formation from [^{14}C]putrescine substrate in control reactions (100%) was 53.94 pmol min^{-1} mg^{-1} protein; specific activity of spermine/thermospermine formation in control reactions (100%) was 5.48 pmol min^{-1} mg^{-1} protein.

Reaction Scheme Using [^{14}C]Putrescine as Initial Substrate in 30-min Reactions	Proportional Amount of Product Formed Relative to Control Reaction	
	Spermidine	Spermine and Thermospermine
	%	
Control, [^{14}C]putrescine for 30 min	100	100
Add 6 mM unlabeled spermidine at 15 min	62	63
Add 6 mM unlabeled putrescine at 15 min	54	75
Add both unlabeled putrescine and spermidine at 15 min	76	70
Add 6 mM unlabeled spermidine at 0 min	90	66
Add both unlabeled putrescine and spermidine at 0 min	79	57
[^{14}C]Spermidine for 30 min (no putrescine)	–	0
[^{14}C]Spermidine +6 mM unlabeled putrescine at 0 min	–	0

based on extrapolation from the smaller polyamines run as standards.

Inhibition of PAPT Activity by the Primary Product, Spermidine

Spermidine has been implicated as being involved in feedback inhibition of plant PAPT (Hartmann et al., 1988). Experiments were designed to test whether alfalfa PAPT in vitro enzyme activity was inhibited by spermidine (Table III). Reactions using [^{14}C]putrescine as the initial substrate were carried out for 30 min. Addition of unlabeled spermidine to the reaction assay at 15 min after initiation of the PAPT reaction (halfway through the reaction period) resulted in a nearly 40% reduction of label conversion from the initial substrate to the major products, spermidine and tetramines. All of the label was counted in these reactions, and volume calculations were adjusted to eliminate the effects of label dilution after the unlabeled spermidine was added to the reactions. The addition of unlabeled spermidine at the beginning of the reaction period resulted in only a 10% reduction in label conversion from the initial substrate to spermidine but a nearly 35% reduction in label conversion to tetramines. Thus, spermidine added to the in vitro reaction assays resulted in the apparent product inhibition of alfalfa PAPT activity.

The addition of unlabeled putrescine 15 min after initiation of the PAPT reaction as a control also resulted in a 25 to 45% reduction in label conversion to products, as expected because of the dilution of labeled substrate with unlabeled substrate and the corresponding dilution of substrate radiolabel available for conversion to products (Table III). The addition of both unlabeled putrescine and unlabeled spermidine, either after 15 min or at the beginning of the reaction period, also resulted in a 20 to 40% reduction in label conversion to products.

Another pair of reaction schemes involved the utilization of [^{14}C]spermidine as the initial substrate instead of radiolabeled putrescine (Table III). The labeled [^{14}C]spermidine substrate reaction failed to result in label conversion to any products, confirming the results presented in Table I. Moreover, addition of unlabeled putrescine at the beginning of the reaction period also failed to result in any label conversion from [^{14}C]spermidine into products. These results confirmed that SAPT activity was not detectable in alfalfa under the conditions of the reported experiments.

DISCUSSION

The pattern of aminopropyltransferase enzyme activity in alfalfa is entirely different from the pattern observed in most bacterial and animal systems. In mammalian systems PAPT and SAPT are known to be two different enzymes, each specific for a unique substrate and producing a single product (Hibasami et al., 1980a; Pegg, 1983). Bacteria have either a single PAPT, because they biosynthesize spermidine as the sole polyamine constituent (Pegg, 1983), or a single aminopropyltransferase with broad substrate specificity, to produce a variety of polyamines (Cacciapuoti et al., 1986). However, aminopropyltransferases have not been well characterized in plants (Flores et al., 1989; Suzuki et al., 1990; Slocum, 1991). From our results it seems that there is only one form of aminopropyltransferase in alfalfa, which is highly specific for putrescine as the initial substrate (Table I). Moreover, a study conducted with an inhibitor that is specific for PAPT but not for SAPT, namely CHA (Hibasami et al., 1980b), demonstrates that this alfalfa enzyme activity represents a classical example of a PAPT (Fig. 2).

There is little direct evidence confirming the presence of SAPT in plants, even though it is generally assumed to be present (Flores et al., 1989; Suzuki et al., 1990). Repeated experiments failed to identify any alfalfa aminopropyltransferase activity that would accept spermidine or any other likely initial substrate (e.g. DAP or spermine) to form additional polyamine products (Tables I and III). The classical approach in mammalian systems has been to supply radiolabeled spermidine as the substrate to detect SAPT activity (Hibasami et al., 1980a; Pegg, 1983), but this same approach failed to yield any products using the alfalfa protein extracts. Thus, there is no SAPT activity, distinct from PAPT, detectable in alfalfa under the conditions used in this study.

Alfalfa PAPT does, however, produce multiple polyamine products. Spermine, thermospermine, and, putatively, homocaldopentamine and homocaldohexamine are

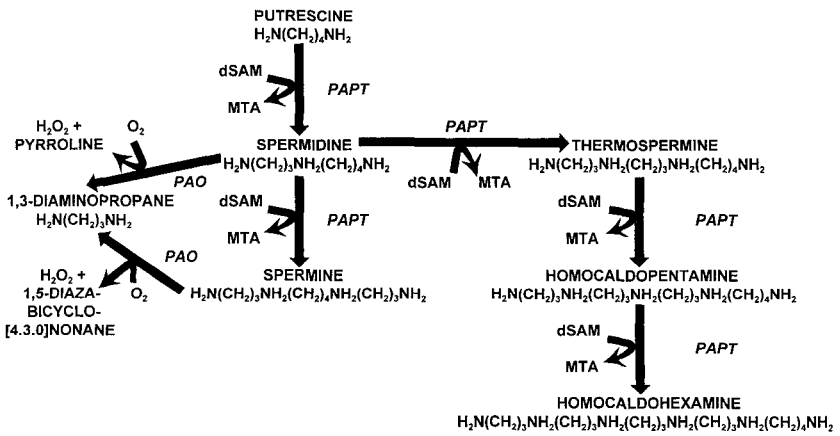

Figure 4. Proposed biosynthetic pathway of polyamines in alfalfa involving PAPT, based on the results of this study. The polyamine oxidase (PAO) branch of the pathway is based on the results of Bagga et al. (1991) and is included here to illustrate comprehensively the known fates of all products of alfalfa PAPT.

all apparent products of alfalfa PAPT, in addition to the expected and predominant product, spermidine (Table I; Figs. 1 and 3). This capacity of alfalfa PAPT to form multiple polyamine products from a single initial substrate makes this enzyme activity unique among the aminopropyltransferases reported to date. There are a few bacteria that exhibit a single aminopropyltransferase capable of producing multiple polyamine products, but that enzyme activity also accepts a corresponding number of different initial substrates (Cacciapuoti et al., 1986), in contrast to the alfalfa enzyme activity reported here.

The initial substrate for PAPT, putrescine, is a diamine interrupted by a butyl group (Fig. 4). Spermidine is formed by the addition of a propylamine group, donated by dSAM, to one end of the putrescine molecule. Spermine is formed by the addition of another propylamine group to spermidine but on the opposing side of the butyl group, resulting in a symmetrical molecule. In contrast, thermospermine is formed by the addition of another propylamine group to spermidine on the same side of the butyl group, resulting in an asymmetrical molecule. Thus, spermine and thermospermine are structural isomers with identical chemical compositions that comigrate during paper electrophoresis and HPLC. We were able to distinguish spermine and thermospermine as distinct alfalfa PAPT reaction products by a TLC method using formaldehyde in the solvent system (Fig. 3). This method is based on TLC separation of various polyamines by Shirahata et al. (1983), and our R_F values of PAPT-produced polyamine products correspond well with standards and previously published results. However, it is not clear whether a single PAPT enzyme carries out the synthesis of both spermine and thermospermine or whether a PAPT enzymatic complex is required to perform the different propylamine addition reactions that result in the formation of spermine and thermospermine. It is clear from our experiments that the formation of these products is enzyme-catalyzed and dependent on dSAM as the propylamine donor.

The other apparent products of alfalfa PAPT activity, homocaldopentamine and homocaldohexamine, are tentatively identified based on their elution behaviors during HPLC and comparison to standards of the common polyamines and synthetic standards of the pentamines. Homocaldopentamine is formed by the addition of a third propylamine group to thermospermine, on the same side of the butyl group as the previous two propylamine groups (Fig. 4). Similarly, homocaldohexamine is formed by the addition of yet another propylamine moiety to homocaldopentamine on the same side of the butyl group. Both compounds are long-chain, asymmetrical molecules.

Results from the time-course experiment (Fig. 1) and from the 1-min reaction sample analyzed by TLC (Fig. 3) show that spermidine is synthesized rapidly from the putrescine substrate. The time-course experiment (Fig. 1) shows that the tetramines spermine and thermospermine are accumulated more slowly than spermidine. However, the 1-min reaction sample analyzed by TLC (Fig. 3) shows that significant spermine is produced relatively quickly, whereas longer reaction times (30–60 min) are required to observe thermospermine formation. The other uncommon polyamine products, putative homocaldopentamine and homocaldohexamine, also require long reaction times for significant accumulation (Table I; Fig. 3), with maximum accumulation occurring at about 60 min. Thus, the initial PAPT enzymatic products, spermidine and spermine, appear to be formed primarily in the first 15 min of the reaction, whereas the uncommon and higher-molecular-weight polyamines are formed as the reaction proceeds over a prolonged time. However, the net accumulation of spermidine and tetramines begins to decline with reaction times of 60 min. Polyamine oxidase is known to be present in alfalfa tissues (Bagga et al., 1991), and oxidation of spermidine and spermine by polyamine oxidase could partially account for their decline after 60 min in the PAPT reaction assays.

Inhibitions of enzymatic activity by CHA (Fig. 2A), which is a specific inhibitor of PAPT, by MTA (Fig. 2B), which is a general inhibitor of aminopropyltransferases, or due to apparent product inhibition by spermidine (Table III), all result in concomitant declines of all enzymatic reaction products at similar rates. Spermidine has been implicated as a feedback inhibitor of plant PAPT in previous work (Hartmann et al., 1988).

Product-inhibition studies (Table III) highlighted the differential rates of product formation observed in the time-course study (Fig. 1) and the TLC results (Fig. 3). Specifically, the addition of unlabeled spermidine halfway through the enzyme reaction period (at 15 min) resulted in a 37 to 38% reduction in the formation of spermidine, as well as the tetramines spermine and thermospermine, when labeled [^{14}C]putrescine was used as the initial substrate (Table III). In contrast, when unlabeled spermidine was added at the time of enzyme reaction initiation (at 0 min), the formation of labeled spermidine from labeled putrescine used as the initial substrate was inhibited by only 10%, whereas the formation of the tetramines spermine and thermospermine continued to be inhibited by 34%. Thus, the addition of an excess of the primary product (unlabeled spermidine) acting as an inhibitor at different times during the enzyme reaction period resulted in differential effects on the rates of product formation. These various observations concerning the differential rates of product formation (Tables I and III; Fig. 3) might be suggestive that an enzyme complex with a single PAPT activity, rather than a single-enzyme molecule, may be responsible for the alfalfa PAPT enzyme activity under study.

Taken together, these results provide the basis for a preliminary description of putative binding sites in the alfalfa PAPT enzyme. The high degree of initial substrate specificity of the alfalfa PAPT suggests that there is a unique site for the binding of putrescine as the sole initial substrate. Although the enzyme accepts the diamine putrescine as the initial substrate, the substrate-binding site is highly discriminatory because it has no apparent binding affinity for a closely related diamine, DAP. Moreover, no apparent binding affinity for the higher-molecular-weight polyamines spermidine and spermine as alternative initial

substrates is observed. The inhibitory action of CHA is probably due to its competitive binding to the putrescine substrate-binding site (Hibasami et al., 1980b; Flores et al., 1989). The inhibitory action of MTA is probably due to competitive binding of MTA to the dSAM propylamine donor-binding site (Hibasami et al., 1980a; Flores et al., 1989). However, the apparent product-inhibitory action of spermidine is not likely to be the result of competitive binding at the putrescine substrate site, because it would be a likely initial substrate for the formation of spermine if this were the case. Radiolabeled [^{14}C]spermidine was not recognized as an initial substrate when used in either the presence or absence of unlabeled putrescine (Tables I and III). Also, the presence of an excess of spermidine resulted in inhibition of alfalfa PAPT enzyme activity. Simple product inhibition often requires a relatively high concentration of the product to observe inhibition of enzyme activity, as was the case in our study. Alternatively, spermidine may be capable of conformationally changing either the putrescine substrate-binding site or the dSAM propylamine donor-binding site sufficiently to result in catalytic inhibition. Whatever the basis of this inhibitory effect of spermidine on alfalfa PAPT, these results are consistent with the conclusion that we are not dealing with a SAPT enzyme activity in the alfalfa system.

Once putrescine is bound to the substrate-binding site, the propylamine addition takes place to form spermidine. Apparently, some proportion of the spermidine product is released from the enzyme as a free product, but some proportion remains bound to the enzyme to form spermine as the result of a further propylamine addition. Presumably, all of the spermine formed is released from the enzyme as a free product. However, because of unknown regulatory features, the spermidine-enzyme complex is capable of forming the alternative tetramine product thermospermine at some later time during the reaction. Some proportion of the thermospermine formed is released from the enzyme as a free product, and some proportion remains bound to the enzyme to form homocaldopentamine by the addition of another propylamine moiety. Similarly, some proportion of the homocaldopentamine product is released as a free product, and some proportion remains bound to the enzyme to form homocaldohexamine by another propylamine addition. Homocaldohexamine is presumably the terminal product of the enzymatic reaction.

The alfalfa PAPT-specific activities for the formation of the common polyamines spermidine and spermine and of uncommon polyamines such as homocaldopentamine, as presented in this paper, are compatible with the data on polyamine titers we presented previously for alfalfa (Kuehn et al., 1990a). However, PAPT does not explain the accumulation of the trimethylenic uncommon polyamines such as norspermidine, norspermine, and caldopentamine (Rodriguez-Garay et al., 1989; Kuehn et al., 1990b). The trimethylenic polyamines are most likely to be biosynthesized from DAP (Fig. 4). An alternate route to formation of spermidine has been identified in *Lathyrus* (Srivenugopal and Adiga, 1980) and in a few bacteria (Tait, 1985), in which L-aspartyl-β-semialdehyde is used as the propylamine donor for the formation of an intermediate (carboxyspermidine), which is then decarboxylated to yield spermidine, as the result of a Schiff base reductase/decarboxylase activity (Adiga and Prasad, 1985). In our recent studies we have been able to show the formation of the uncommon polyamines norspermidine, norspermine, caldopentamine, and caldohexamine in a reaction utilizing radiolabeled DAP as the substrate and L-aspartyl-β-semialdehyde as the propylamine donor, as the result of a Schiff base reductase/decarboxylase-like activity (S. Bagga, G. Kuehn, and G. Phillips, unpublished results). The details of this alternative polyamine biosynthetic enzyme activity will be published in a separate manuscript.

In earlier studies (Rodriguez-Garay et al., 1989; Kuehn et al., 1990a) we reported the accumulation of significant amounts of uncommon polyamines in osmotic stress-tolerant cultivars of alfalfa exposed to water-deficit conditions. Our present study shows that PAPT is responsible for producing both spermidine and tetramines (spermine and thermospermine) in both the osmotic stress-susceptible (Mesilla-0) and stress-tolerant (Mesilla-3) cultivars of alfalfa (Table II). However, the uncommon and high-molecular-weight polyamines tentatively identified as homocaldopentamine and homocaldohexamine were produced only in significant amounts by enzyme extracts from the stress-tolerant (Mesilla-3) cultivar challenged with water-deficit stress. The PAPT activity was strongest in meristematic shoot tips and immature floral buds. We failed to detect significant levels of PAPT activity in roots, fully expanded leaves, and stem internodes, although meristematic root tips were not analyzed separately from the remainder of the root system. Seedling tissues showed low levels of PAPT activity (Table II). It is possible that the alfalfa PAPT enzyme is activated under osmotic stress conditions to produce the uncommon polyamines, possibly as a long-term mechanism of protection against stress. It is plausible to suggest that the PAPT activity and the accumulation of the possibly protective polyamines would be favored in meristematic tissues that are needed for plant recovery following stress and are not required in more mature nonmeristematic tissues that will not be utilized or will not be reactivated for growth during plant recovery from stress.

Based on our results and the interpretations presented above, we propose the pathway depicted in Figure 4 to describe the role of PAPT in the biosynthesis of several common and uncommon polyamines in alfalfa. This pathway accounts for all of the characteristics of alfalfa PAPT activity as revealed in our studies, as well as the fate of all of its products. Figure 4 also includes the known mechanism of oxidation of spermidine and spermine into DAP by polyamine oxidase (Bagga et al., 1991). A central feature of this pathway is the implication that spermidine is partitioned into as many as three different polyamine products, spermine, thermospermine, and DAP. Directions for future study may include investigations of the regulatory features of PAPT activity, the overall regulatory control over polyamine accumulation in plants, and the precise physiological role(s) played by the polyamines, e.g. during abiotic stress responses.

ACKNOWLEDGMENTS

The authors thank Dr. Cliff Currier and Shaun Townsend for their assistance with growing plant materials in a rainout shelter, Professor Koei Hamana for the gift of the synthetic uncommon polyamine samples, and Emine Koc for her assistance with HPLC analyses.

Received September 11, 1996; accepted February 22, 1997.
Copyright Clearance Center: 0032–0889/97/114/0445/10.

LITERATURE CITED

Adiga PR, Prasad GL (1985) Biosynthesis and regulation of polyamines in higher plants. Plant Growth Regul **3:** 205–226

Bagga S, Dharma A, Phillips GC, Kuehn GD (1991) Evidence for the occurrence of polyamine oxidase in the dicotyledonous plant *Medicago sativa* L. (alfalfa). Plant Cell Rep **10:** 550–554

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Cacciapuoti G, Porcelli M, Carteni-Farina M, Gambacorta A, Zappia V (1986) Purification and characterization of propylamine transferase from *Solfolobus solfataricus*, an extreme thermophilic archaebacterium. Eur J Biochem **161:** 263–271

Flores HE, Protacio CM, Signs MW (1989) Primary and secondary metabolism of polyamines in plants. Rec Adv Phytochem **23:** 329–393

Fujita K, Magatsu T, Shinpo K, Maruta K, Teradaira R, Nakamura M (1980) Improved analysis for urinary polyamines by use of high voltage electrophoresis on paper. Clin Chem **26:** 1577–1582

Hartmann TH, Sander H, Adolph R, Toppel G (1988) Metabolic links between the biosynthesis of pyrrolizidine alkaloids and polyamines in root cultures of *Senecio vulgaris*. Planta **175:** 82–90

Hibasami H, Borchardt RT, Chen SY, Coward JK, Pegg AE (1980a) Studies of inhibition of rat spermidine synthase and spermine synthase. Biochem J **187:** 419–428

Hibasami H, Tanaka M, Nagai J, Ikeda K (1980b) Dicyclohexylamine, a potent inhibitor of spermidine synthase in mammalian cells. FEBS Lett **116:** 99–101

Hirasawa E, Suzuki Y (1983) Biosynthesis of spermidine in maize seedlings. Phytochemistry **22:** 103–106

Kuehn GD, Bagga S, Rodriguez-Garay B, Phillips GC (1990a) Biosynthesis of uncommon polyamines in higher plants and their relation to abiotic stress responses. *In* HE Flores, RN Arteca, JC Shannon, eds, Polyamines and Ethylene: Biochemistry, Physiology, and Interactions. American Society of Plant Physiologists, Rockville, MD, pp 190–202

Kuehn GD, Rodriguez-Garay B, Bagga S, Phillips GC (1990b) Novel occurrence of uncommon polyamines in higher plants. Plant Physiol **92:** 88–96

Lee SH, Park KY (1991) Compensatory aspects of the biosynthesis of spermidine in tobacco cells in suspension culture. Plant Cell Physiol **32:** 523–531

Margosiak SA, Dharma A, Gonzales AP, Louie D, Kuehn GD (1990) Identification of the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase as a substrate for transglutaminase in plants. Plant Physiol **92:** 88–96

Niitsu M, Samejima K (1986) Synthesis of a series of linear pentaamines with three and four methylene chain intervals. Chem Pharm Bull (Tokyo) **34:** 1032–1038

Pegg AE (1983) Assay of aminopropyltransferases. Methods Enzymol **94:** 260–265

Phillips GC, Kuehn GD (1991) Uncommon polyamines in plants and other organisms. *In* RD Slocum, HE Flores, eds, Biochemistry and Physiology of the Polyamines in Plants. CRC Press, Boca Raton, FL, pp 121–136

Rodriguez-Garay B, Phillips GC, Kuehn GD (1989) Detection of norspermidine and norspermine in *Medicago sativa* L. (alfalfa). Plant Physiol **89:** 525–529

Shirahata A, Takeda Y, Kawase M, Samejima K (1983) Detection of spermine and thermo-spermine by thin-layer chromatography. J Chromatogr **262:** 451–454

Sindhu RK, Cohen SS (1984a) Subcellular localization of spermidine synthase in the protoplasts of Chinese cabbage leaves. Plant Physiol **76:** 219–223

Sindhu RK, Cohen SS (1984b) Propylamine transferases in Chinese cabbage leaves. Plant Physiol **74:** 645–649

Slocum RD (1991) Polyamine biosynthesis in plants. *In* RD Slocum, HE Flores, eds, Biochemistry and Physiology of Polyamines in Plants. CRC Press, Boca Raton, FL, pp 23–40

Srivenugopal KS, Adiga PR (1980) Coexistence of two pathways of spermidine biosynthesis in *Lathyrus sativus* seedlings. FEBS Lett **112:** 260–264

Suzuki Y, Hirasawa E, Yanagisawa H, Matsuda H (1990) The enzymes of polyamine metabolisms in higher plants. *In* HE Flores, RN Arteca, JC Shannon, eds, Polyamines and Ethylene: Biochemistry, Physiology, and Interactions. American Society of Plant Physiologists, Rockville, MD, pp 73–90

Tabor CW, Tabor H (1983) Putrescine aminopropyltransferase (*Escherichia coli*). Methods Enzymol **94:** 265–270

Tait GH (1985) Bacterial polyamines, structures and biosynthesis. Biochem Soc Trans **13:** 316–318

Wickner RB, Tabor CW, Tabor H (1970) Purification of *S*-adenosylmethionine decarboxylase from *Escherichia coli* W: evidence for covalently bound pyruvate. J Biol Chem **245:** 2132–2139

Yamanoha B, Cohen SS (1985) S-Adenosylmethionine decarboxylase and spermidine synthase from Chinese cabbage. Plant Physiol **78:** 784–790