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Propylamine Transferases in Chinese Cabbage Leaves[1]

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ABSTRACT

We have found spermidine synthase and spermine synthase activities in extracts of leaves of Chinese cabbage (*Brassica pekinensis* var. Pak Choy) and have developed an assay of the former in crude extracts. The method is based on the transfer of the propylamine moiety of decarboxylated *S*-adenosylmethionine to labeled putrescine, followed by ion-exchange separation of the labeled amine substrate and product, which are then converted to the 5-dimethylamino-1-napthalene sulfonyl (dansyl) derivatives and further purified and identified by thin layer chromatography. The specific radioactivity of putrescine present in the reaction mixture is determined, as is the radioactivity present in dansyl spermidine. The enzyme is also present in extracts of spinach leaves.

Spermidine synthase has been purified about 160-fold from Chinese cabbage leaves. After partial purification, a rapid coupled enzymic assay has been used to study various properties of the enzyme. The plant enzyme shows maximum activity at pH 8.8 in glycine-NaOH buffer and has a molecular weight of 81,000. The K_m values for decarboxylated *S*-adenosylmethionine and putrescine are 6.7 and 32 micromolar, respectively. The enzyme activity is inhibited strongly by dicyclohexylamine, cyclohexylamine, and *S*-adenosyl-3-thio-1, 8-diaminoctane. Of these, dicyclohexylamine is the most potent inhibitor with an I_{50} at 0.24 micromolar.

Propylamine transferases, catalyzing the synthesis of spermidine from putrescine (spermidine synthase, EC 2.5.1.16) and of spermine from spermidine (spermine spermidine, EC 2.5.1.-), utilize decarboxylated SAM[2] as the propylamine donor. Both the polyamine and MTA are formed in equivalent amounts. Spermidine synthase was first discovered in *Escherichia coli* (23) and was later purified and characterized from this source (2). Spermidine synthase and spermine synthase have been reported in several mammalian tissues (reviewed in 26) and are separable from SAM decarboxylase. With the aid of affinity chromatography, these enzymes have now been purified to homogeneity from rat ventral prostate and bovine brain and characterized (16, 17).

In a plant, putrescine may be formed by one of several routes and the use of one or another route has been investigated (reviewed in 20). Although a path which bypasses methionine has been described (21, 24), we have found that methionine is a precursor of spermidine in cyanobacteria and of spermidine and spermine in Chinese cabbage (3, 4, 8). We have also shown the presence of spermidine synthase in healthy and turnip yellow mosaic virus-infected Chinese cabbage leaves and leaf protoplasts (3, 4) and are now attempting to relate the enzyme activity to the physiological roles of spermidine in normal and virus-infected plant cells. During the virus infection, spermidine accumulates to several times the normal level (25), and a significant fraction of the triamine is found in a nonexchangeable association with the virus (5).

Despite the advances with the bacterial and mammalian enzymes, there have been few studies on the enzymes of spermidine and spermine synthesis in plants. Decarboxylated SAM, the propylamine donor, is not commercially available and has to be prepared either enzymically or synthesized chemically. Furthermore, the crude plant extracts are complex, which limit the choice of a suitable assay of the enzymes. Methods dependent on the isolation of labeled MTA from labeled decarboxylated SAM (10) could not be applied to the Chinese cabbage extracts because MTA was rapidly degraded. Coupled enzymic assays (2, 22, 27) are affected adversely by the presence of degradative enzymes. Bypassing these difficulties, we have developed an assay for spermidine synthase which is useful for crude extracts. After partial purification of the enzyme, a rapid coupled enzymic assay (19, 22) can be applied.

MATERIALS AND METHODS

Chemicals. Putrescine·2HCl, spermidine·3HCl, and spermine·4HCl were purchased from Calbiochem. Commercial samples of spermidine were recrystallized before use. Labeled polyamines were purchased from New England Nuclear. The labeled spermidine, which contained small but significant amounts of labeled spermine was purified by ion-exchange chromatography. Dowex-50W (H^+) (200–400 mesh, 8% crosslinked) was from Bio-Rad Laboratories. Horseradish peroxidase (type II), homovanillic acid, dicyclohexylammonium sulfate, and MTA were obtained from Sigma. Cyclohexylamine was from Pfaltz and Bauer, Inc. Oat seedling polyamine oxidase was prepared as described by Suzuki *et al.* (22).

Decarboxylated SAM was prepared either from [1-^{14}C]- or [^{3}H-methyl]SAM with the aid of partially purified SAM decarboxylase from *E. coli* (3). Preparations of synthetic decarboxylated SAM were kindly provided to us by Dr. K. Samejima of Tokyo. *S*-Adenosyl-3-thio-1,8-diaminooctane and 5′-methylthiotubercidin were generous gifts from Dr. James K. Coward of Rensselaer Polytechnic Institute, Troy, NY. All other chemicals were of the highest purity available commercially.

Plant Material. Chinese cabbage (*Brassica pekinensis* var. Pak Choy) plants were grown in a controlled environment chamber as described earlier (3). When the plants were about 3 to 6 weeks old, young leaves (<5 cm long) were used for the preparation of extracts. Full grown spinach leaves were obtained from a local farm stand.

Preparation of Enzyme Extracts. The cabbage leaves (5 g) were homogenized with 10 mM Gly-NaOH, pH 8.8 with a pestle and mortar at 4°C. The homogenate was passed through eight layers

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[2] Abbreviations: SAM, *S*-adenosylmethionine; MTA, 5′-deoxy-5′-methylthioadenosine; PCA, perchloric acid; dansyl, 5-dimethylamino-1-naphthalene sulfonyl.

of cheesecloth and adjusted to 10 ml. A portion of this crude extract was centrifuged at 10,000*g* for 20 min at 4°C to obtain a supernatant fraction. Spinach leaves were washed, blotted dry, deribbed, and weighed. The leaves (5 g) were ground at 4°C in 25 mM sodium phosphate, pH 7.2, and the extract was passed through cheesecloth and adjusted to 10 ml.

Enzyme Assays: In estimating the propylamine transferases in the crude extracts, the reaction mixture contained 150 mM Gly-NaOH buffer, pH 8.8, 37 μM [^{14}C]putrescine·2HCl (102 mCi/mmol) for spermidine synthase, or 37 μM [^{14}C]spermidine·3HCl (98.7 mCi/mmol) for spermine synthase, 25 μM decarboxylated SAM, an appropriate amount of enzyme (0.03–8.0 units), and H_2O in a total volume of 0.325 ml. Since only 50% of the synthetic decarboxylated SAM was active in the reaction (15, unpublished data), the concentrations of this compound given in this paper are divided by 2 to reflect the concentrations of the active component. Control mixtures lacked either decarboxylated SAM or the enzyme extract. The tubes were incubated at 37°C for 1 h and the reaction was stopped by adding 1 ml of cold 5% PCA. After standing 30 min at 4°C, the mixtures were centrifuged. The precipitates were washed twice with 1 ml each of cold 3% PCA, and supernatant fluids were combined and processed as described in the next section.

For studies with the partially purified spermidine synthase, the fluorometric assay of Suzuki *et al.* (22) was used. The standard assay mixture contained 33 μM potassium phosphate buffer, pH 7.4, 0.04 ml of the partially purified enzyme, 0.05 ml of oat seedling polyamine oxidase solution (1 mg/ml), 0.05 ml of horseradish peroxidase solution (1 mg/ml), 0.05 ml of homovanillic acid solution (1 mg/ml), 100 μM putrescine·2HCl, 25 μM decarboxylated SAM, and H_2O in a total volume of 0.6 ml. The reaction was started by adding decarboxylated SAM. After incubation at 37°C for 1 h, the enzyme reaction was stopped by adding 0.05 ml of 1 N NaOH. The tubes were cooled, and the fluorescence intensity was measured with excitation at 323 nm and emission at 426 nm in a spectrofluorometer Mark I (Farrand Optical Co.). Control mixtures were set up lacking decarboxylated SAM. The reaction was linear from 1.0 to 10.0 nmol of spermidine. Under these conditions also, the amount of spermidine formed was proportional to the amount of partially purified enzyme protein up to about 75% of maximal spermidine production. At a spermidine yield of 40% of maximum in 1 h, spermidine production continued at a constant rate for an additional hour before the rate decreased. The protein in a crude extract or in a supernatant fraction was estimated by the method of Lowry *et al.* (13) after precipitation with cold TCA and two washings with cold acetone:methanol (7:2, v/v) to eliminate interfering Chl.

One unit of enzyme is defined as the amount of enzyme required to form 1 nmol of spermidine or spermine/h under the standard assay conditions.

Quantification of the Polyamines. Dansyl derivatives of polyamines were prepared and quantified by TLC essentially as described (3).

RESULTS

Selective Removal of the Unused Substrates. The reactions were stopped at 10% or less of the theoretical maximum, *i.e.* 8 nmol or the content of active decarboxylated SAM, leaving a radioactive substrate in amounts greater than 15 times that of the labeled product. It was necessary to remove most of the substrate prior to dansylation and chromatographic separation of the labeled product to minimize the contamination of the dansylated product by traces of the dansylated precursor. Although a chromatographic solvent chloroform:triethylamine (80:16, v/v) was used in TLC in which the dansyl product migrated ahead of the dansyl substrate, this did not entirely eliminate the problem. Thus, putrescine trailing to the spermidine position or spermidine to the spermine position was at least 2% in the ethyl acetate:cyclohexane (1:2, v/v) solvent, whereas the carrying forward of putrescine to spermidine or of purified spermidine to spermine was only about 0.7% in the chloroform-triethylamine solvent.

To the combined supernatants, derived from the PCA precipitation of the assay tubes, were added 30 nmol of unlabeled spermidine or spermine as carriers, and the solutions were loaded onto 1 × 5 cm columns of Dowex-50W (H^+), prepared as described by Inoue and Mizutani (12). The columns were then washed with 40 ml of 0.1 M sodium phosphate buffer, pH 8.0, containing 0.7 M NaCl, 10 ml of 1 M HCl, and 40 ml of 2.3 M

Table I. *Separation of a Mixture of [^{14}C]Putrescine and [^{3}H] Spermidine on a Dowex-50W (H^+) Column*

The mixture applied to the column in 3% PCA contained 30 nmol of each of the two labeled polyamines.

	[^{14}C]Putrescine		[^{3}H]Spermidine	
	cpm	*% of total*	*cpm*	*% of total*
Effluent	6	0.1	41	0.5
Sodium phosphate buffer wash	303	4.5	322	3.9
1 M HCl wash	68	1.0	60	0.7
2.3 M HCl elutate	6212	92.5	465	5.7
6 M HCl eluate	125	1.9	7322	89.2

Table II. *Separation of a Mixture of [^{3}H]Spermidine and [^{14}C] Spermine on a Dowex-50W (H^+) Column*

The mixture applied to the column in 3% PCA contained 30 nmol of each of the two labeled polyamines.

	[^{3}H]Spermidine		[^{14}C]Spermine	
	cpm	*% of total*	*cpm*	*% of total*
Effluent	52	1.1	41	0.6
Sodium phosphate buffer wash	168	3.5	155	2.1
1 M HCl wash	18	0.4	69	0.9
3.3 M HCl eluate	4478	94.3	251	3.4
6 M HCl eluate	31	0.7	6764	92.9

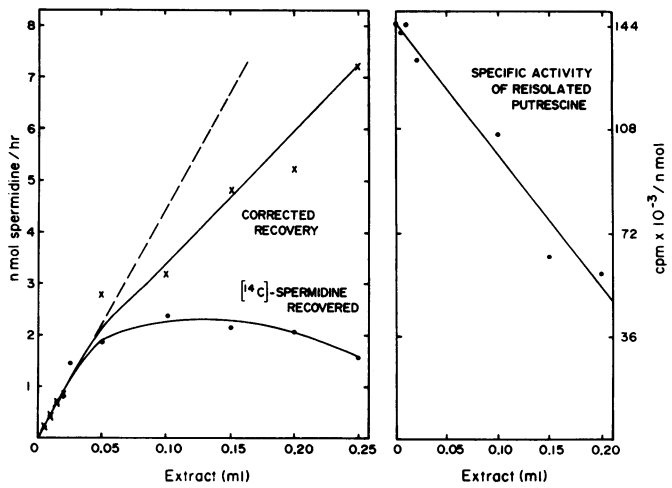

FIG. 1. Assay of spermidine synthase in a supernatant fraction of a crude cabbage leaf extract. Righthand chart indicates the decrease of specific radioactivity of [^{14}C]putrescine after dilution by the extract. Lefthand chart presents radioactive spermidine recovered and its molar equivalent after correction for the specific radioactivity of putrescine in the reaction mixture. The fraction contained 8.0 mg protein/ml.

Table III. *Presence of Spermine Synthase in the Crude Extract and Supernatant Fraction of Chinese Cabbage Leaves*

The enzyme activity was assayed at 37°C.

Assay System	Total Radioactivity	Enzyme Units/mg Fresh Tissue	Enzyme Units/mg Protein
	cpm × 10^{-3}		
Crude extract	19.9	0.90	0.05
Minus enzyme extract	2.0		
Minus decarboxylated SAM	2.1		
Heated extract	2.0		
Supernatant fraction	26.2	1.20	0.08

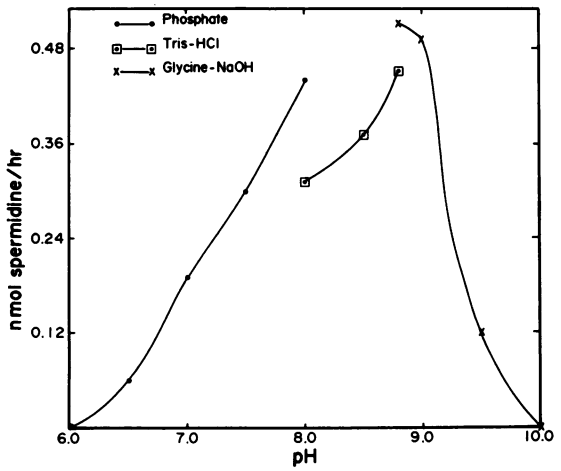

FIG. 2. Effect of pH on spermidine synthase activity. The enzyme activity was assayed at various pH using labeled putrescine as the substrate.

Table IV. *Inhibition of the Cabbage Spermidine Synthase*

Compound	Concentration Required for 50% Inhibition
	μM
Dicyclohexylamine	0.24
Cyclohexylamine	0.54
S-Adenosyl-3-thio-1,8 diaminooctane	1.3
5′-Deoxy-5′-methylthiotubercidin	110
5′-Deoxy-5′-methylthioadenosine	210

HCl for spermidine synthase or 3.3 M HCl for spermine synthase. The labeled reaction products were eluted with 30 ml of 6 M HCl. The selective removal of 93% of the [^{14}C]putrescine from spermidine is shown in Table I. About 90% of the spermidine was retained onto the column and could be eluted with 6 M HCl.

The selective removal of spermidine from spermine with 3.3 M HCl in the assay of spermine synthase is shown in Table II. More than 99% of spermidine was removed in this way; the residual 93% of the spermine still on the column could be eluted with 6 M HCl. Molarities of HCl less than 3.3 removed spermidine poorly, *i.e.* 2.6 and 2.9 M HCl removed only 7.3 and 14% of spermidine, respectively. Higher molarities of HCl, *i.e.* 3.5, 3.8, and 4.1 M HCl, were found to elute 16.6, 67.5, and 98% spermine, respectively.

The eluates at 2.3 M and 3.3 M HCl are suitable for the isolation of dansyl putrescine and dansyl spermidine, respectively, as described below. After these separations the specific radioactivities of the substrates for spermidine synthase and of spermine synthase can be estimated, as well as the radioactivities of the products.

Identification of the Reaction Product by TLC. The 6 M HCl eluates containing the labeled products were dried at 45°C *in vacuo*, dissolved in 0.2 ml of 30 mM HCl, dansylated, and analyzed as described earlier (3). The plate was developed once in chloroform:triethylamine (80:16, v/v), a solvent found to permit a complete separation of small amounts of the di-, tri-, and tetramines. The spots co-migrating with authentic dansyl spermidine and dansyl spermine were scraped and counted in 1 ml H_2O and 10 ml Aquasol-2 (New England Nuclear). The recoveries of dansyl spermidine and dansyl spermine from the plate were 82 and 86%, respectively.

Effect of Extract Concentration on the Apparent Activity of Spermidine Synthase. The effect of varying the concentration of the various extracts on the recovery of radioactive spermidine revealed an apparent maximal yield of 30%. Linearity of spermidine formed as a function of the volume of the supernatant fraction of the cabbage extract added was found only at small amounts of extract (Fig 1). The calculation of spermidine actually made depends on the assumed specific activity of labeled putrescine. When the specific radioactivity of putrescine was determined after termination of the reaction, it was found to have decreased considerably as a function of the volume of supernatant added, as presented in Figure 1. Correction of the yields of spermidine markedly increased the apparent activity, with the rates falling relatively little after about a 25% formation of product. The latter figure is calculated from the limiting amount of decarboxylated SAM (8 nmol) added to the system. In addition to the absence of major product inhibition in the system, these data and the corrected values reveal that there had been little if any *de novo* synthesis of putrescine and degradation of added decarboxylated SAM.

Presence of Spermidine Synthase in the Crude Extracts of Spinach Leaves. Spinach leaves were purchased from a local farm stand and the enzyme activity was assayed as described in "Materials and Methods." A crude extract of mature spinach leaves contained about 60% of the spermidine synthase activity compared to that found in actively growing cabbage leaves. Since spinach is more easily available compared to Chinese cabbage, spinach leaves can be a major source of the enzyme.

Presence of Spermine Synthase in the Crude Extracts and Supernatant Fractions of Cabbage Leaves. The presence of spermine synthase in the crude extract and supernatant fraction of Chinese cabbage leaves is shown in Table III. As presented in the Table, the enzyme activity is slightly but reproducibly higher in the supernatant fraction compared to that in the crude extracts.

Partial Purification of Spermidine Synthase from Chinese Cabbage Leaves. The enzyme has been purified about 160-fold from actively growing cabbage leaves (18). Briefly, the supernatant fraction was adjusted to pH 5.1 with acetic acid and precipitated with streptomycin sulfate. After centrifugation, the supernatant was fractionated with $(NH_4)_2SO_4$ at pH 8.8. The active fraction, precipitated between 33 to 65% $(NH_4)_2SO_4$ saturation, was dissolved in a one-tenth volume of 25 mM sodium phosphate, pH 7.2. An equal volume of acetone (−20°C) was then added slowly with stirring and the heavy precipitate was discarded. Three additional volumes of acetone were added and the precipitate was centrifuged and dissolved in 25 mM sodium phosphate, pH 7.2, containing 0.1 M KCl. All steps to this stage can be completed within a day. The coupled assay can be applied to the redissolved acetone precipitate and to all fractions after this stage. Fractionation on a Sephadex G-100 column equilibrated with 25 mM sodium phosphate, pH 7.2, containing 0.1 M KCl, led to elution of the enzyme activity in a single sharp peak. The enzyme preparation thus obtained was stored frozen at −20°C in small aliquots.

Stability. The plant enzyme is very labile in the crude extracts.

More than 90% of the activity was lost after a week when the crude extracts were stored frozen at −15°C (3). The enzyme in crude extracts was also rapidly inactivated during dialysis against Gly-NaOH buffer at pH 8.8. Introduction of 1 mM DTT and/or 1 mM EDTA in the dialysis medium did not prevent this loss in the enzyme activity. Addition of the heated supernatant of an undialyzed enzyme preparation also failed to restore the activity of the cabbage enzyme. No enzyme activity was found in dialyzed crude extracts in the coupled assay of Suzuki *et al.* (22). However, after the acetone precipitation, the enzyme could be stored frozen at −20°C for at least 3 months. After the final filtration through Sephadex, the enzyme lost about 30% activity after a month, when stored frozen at −20°C.

Molecular Weight. The estimation of mol wt of the cabbage spermidine synthase was made with a calibrated column of Sephadex G-100 used for the final step of purification. The column was calibrated with BSA, ovalbumin, α-chymotrypsinogen, and Cyt *c* as described by Andrews (1). The apparent mol wt of the plant enzyme was estimated at about 81,000. The mol wt of mammalian and bacterial spermidine synthases has been reported to be 73,000 (2, 17).

Kinetic Properties of the Enzyme. The effect of pH on spermidine synthase was studied between pH 6.0 and 10.0 using phosphate, Tris-HCl and Gly-NaOH buffers. The plant enzyme showed maximum activity at pH 8.8 in Gly-NaOH buffer (Fig. 2). At a pH more than 9.0 in Gly-NaOH buffer, the enzyme was rapidly inactivated and no activity could be found at pH 10.0. The activity of the plant enzyme was generally lower in Tris buffer than that found in either phosphate or Gly-NaOH buffer at the same pH. The pH optimum for the bacterial (2) and mammalian (17) spermidine synthases have been shown to be 10.4 and 10.0, respectively.

The effect of decarboxylated SAM concentration on the activity of the cabbage enzyme was studied by varying the concentration of this nucleoside from 4 to 50 μM at a fixed putrescine concentration of 1 mM. The K_m value for decarboxylated SAM, determined by reciprocal plots, was 6.7 μM. Decarboxylated SAM at concentrations more than 50 μM was found to be highly inhibitory. The apparent K_m value for putrescine (between 25 and 250 μM) at a fixed concentration of decarboxylated SAM (25 μM) was about 32 μM. The K_m values for putrescine and decarboxylated SAM for spermidine synthases from other sources vary according to different workers (2, 7, 14–16).

Inhibitors. The effect of various inhibitor concentrations was studied under the standard assay conditions. Our results confirmed earlier reports (7, 9), that spermidine synthase is strongly inhibited at higher concentrations of decarboxylated SAM. It has also been reported that the apparent K_m values for putrescine vary greatly according to the concentration of decarboxylated SAM used as the substrate (9). For these reasons, we have defined the relative activities of the inhibitors in terms of I_{50}, *i.e.* the concentration of inhibitor giving 50% inhibition of the enzyme activity. The inhibitory effect of these compounds was dependent on the log of concentration as a smooth S-shaped curve (data not shown). It can be seen in Table IV that the activity of the plant spermidine synthase was inhibited by dicyclohexylamine, cyclohexylamine, and *S*-adenosyl-3-thio-1,8-diaminooctane. Less potent inhibitors included 5′-deoxy-5′-methylthiotubercidin and 5′-deoxy-5′-methylthioadenosine. Of the inhibitors tested, dicyclohexylamine was found to be the most potent with an I_{50} at 0.24 μM.

DISCUSSION

Two separable propylamine transferases requiring decarboxylated SAM as the propylamine donor are present in mammalian cells. In this communication, we have shown the presence of spermidine synthase and spermine synthase in Chinese cabbage leaves. A method is presented for the rigorous assay of spermidine synthase in crude or clarified extracts which involves the use of a radioactive naturally occurring polyamine. The normal levels of these compounds dilute the radioactive substrate considerably, a situation which may exist in the assay of some other plant enzymes, *e.g.* arginine decarboxylase. The resulting dilution of substrate thereby compels the estimation of the specific radioactivity of the labeled substrate present in the reaction mixture. When the molar yield of radioactive spermidine is determined with the aid of this value, the assay approaches linearity over the entire range of extract used and is rigorously proportional to extract for about 25% of the possible synthesis.

The presence of nonradioactive spermidine in the extract probably presents a more serious problem for the assay of spermine synthase with radioactive spermidine as the substrate. In Chinese cabbage leaves, spermidine is present at 5 to 10 times the concentration of putrescine. However, unlike putrescine, which is readily exchangeable from many nucleates and other anionic polymers, much spermidine associated with cell structure probably does not exchange readily. For this reason, it is not as yet possible to determine the dilution of labeled spermidine in a crude extract. If exchangeable spermidine does dilute radioactive substrate in the crude extract, the values reported in Table III will be an underestimate of spermine synthase present in the cabbage leaves.

The improved separation of substrate and product and the introduction of the step of estimating the specific activity of putrescine will improve the assay of spermidine synthase in crude extracts of various cells and tissues rich in putrescine, such as *E. coli*, some animal cells, or in virus-infected plant protoplasts (3). Because of difficulties in the assay of spermidine synthase, the increased recognition of the importance of the polyamines and spermidine in particular has not led to a broad examination of the regulation of this enzyme. However, it may be anticipated that the increased availability of decarboxylated SAM and labeled polyamines, as well as the resolution of the various problems of assay, will result in increased studies of the enzyme in tumorigenesis, virus infection, and senescence.

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LITERATURE CITED

1. ANDREWS P 1964 Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem J 91: 222–233
2. BOWMAN WH, CW TABOR, H TABOR 1973 Spermidine biosynthesis. Purification and properties of propylamine transferase from *Escherichia coli*. J Biol Chem 248: 2480–2486
3. COHEN SS, R BALINT, RK SINDHU 1981 The synthesis of polyamines from methionine in intact and disrupted leaf protoplasts of virus-infected Chinese cabbage. Plant Physiol 68: 1150–1155
4. COHEN SS, R BALINT, RK SINDHU, D MARCU 1981 Polyamine biosynthesis and metabolism in normal and virus-infected plant protoplasts. Med Biol 59: 394–402
5. COHEN SS, ML GREENBERG 1981 Spermidine, an intrinsic component of turnip yellow mosaic virus. Proc Natl Acad Sci USA 78: 5470–5474
6. COHEN SS, DE MARCU, RF BALINT 1981 Light-dependent fixation of polyamines into chloroplasts of Chinese cabbage. FEBS Lett 141: 93–97
7. COWARD JK, NC MOTOLA, JD MOYER 1977 Polyamine biosynthesis in rat prostate. Substrate and inhibitor properties of 7-deaza analogues of decarboxylated *S*-adenosylmethionine and 5′-methylthioadenosine. J Med Chem 20: 500–505
8. GUARINO LA, SS COHEN 1979 The estimation of turnover of spermidine in *Anacystis nidulans*. Anal Biochem 95: 73–76
9. HIBASAMI H, RT BORCHARDT, SY CHEN, JK COWARD, AE PEGG 1980 Studies on inhibition of rat spermidine synthase and spermine synthase. Biochem J 187: 419–428
10. HIBASAMI H, AE PEGG 1978 Rapid and convenient method for the assay of aminopropyltransferases. Biochem J 169: 709–712
11. HIBASAMI H, M TANAKA, J NAGAI, T IKEDA 1980 Dicyclohexylamine, a potent inhibitor of spermidine synthase in mammalian cells. FEBS Lett 116: 99–

101
12. INOUE H, A MIZUTANI 1973 A new method for isolation of polyamines from animal tissues. Anal Biochem 56: 408–416
13. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
14. PEGG AE, HG WILLIAMS-ASHMAN 1969 On the role of *S*-adenosylmethionine in the biosynthesis of spermidine by rat prostate. J Biol Chem 244: 682–693
15. SAMEJIMA K, Y NAKAZAWA 1980 Action of decarboxylated *S*-adenosylmethionine analogues in spermidine-synthesizing system from rat prostate. Arch Biochem Biophys 201: 241–246
16. SAMEJIMA K, A RAINA, B YAMANOHA, T ELORANTA 1983 Purification of putrescine aminopropyltransferase (spermidine synthase) from eukaryotic tissues. Methods Enzymol 94: 270–276
17. SAMEJIMA K, B YAMANOHA 1982 Purification of spermidine synthase from rat ventral prostate by affinity chromatography on immobilized *S*-adenosyl (5′)-3-thiopropylamine. Arch Biochem Biophys 216: 213–222
18. SINDHU RK, SS COHEN 1983 Spermidine synthase of Chinese cabbage. Methods Enzymol 94: 279–285
19. SMITH TA 1976 Polyamine oxidase from the leaves of cereals. Biochem Soc Trans 4: 74–77
20. SMITH TA 1981 Biosynthesis and metabolism of polyamines in plants. *In* DR Morris, LJ Marton, eds, Polyamines in Biology and Medicine. Marcel Dekker, Inc., New York, pp 77–82
21. SRIVENUGOPAL KS, PR ADIGA 1980 Coexistence of two pathways of spermidine biosynthesis in *Lathyrus sativus* seedlings. FEBS Lett 112: 260–264
22. SUZUKI O, T MATSUMOTO, M OYA, Y KATSUMATA, K SAMEJIMA 1981 A new fluoremetric assay for spermidine synthase. Anal Biochem 115: 72–77
23. TABOR H, SM ROSENTHAL, CW TABOR 1958 The biosynthesis of spermidine and spermine from putrescine and methionine. J Biol Chem 233: 907–914
24. TAIT GH 1976 A new pathway for the biosynthesis of spermidine. Biochem Soc Trans 4: 610–612
25. TORGET R, L LAPI, SS COHEN 1979 Synthesis and accumulation of polyamines and *S*-adenosylmethionine in Chinese cabbage infected by turnip yellow mosaic virus. Biochem Biophys Res Commun 87: 1132–1139
26. WILLIAMS-ASHMAN HG, AE PEGG 1981 Aminopropyl group transfers in polyamine biosynthesis. *In* DR Morris, LJ Marton, eds, Polyamines in Biology and Medicine. Marcel Dekker, Inc., New York, pp 43–73
27. ZAPPIA V, M CARTENI-FARINA, P GALLETTI 1977 Adenosylmethionine and poly amine biosynthesis in human prostate. *In* F Salvatore, E Borek, V Zappia, HG Williams-Ashman, eds, The Biochemistry of Adenosylmethionine. Columbia University, New York, pp 473–492