α-Methylated Polyamines as Potential Drugs and Experimental Tools in Enzymology

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Abstract: We describe synthesis of α-methylated analogues of the natural polyamines and their use as tools in unraveling polyamine functions. Experiments with α-methylated spermidine and spermine revealed that the polyamines are exchangeable in supporting cellular growth. Degradation of the analogues by polyamine oxidase disclosed hidden, aldehyde-guided stereospecificity of the enzyme.

Key Words: Hypusine, liver regeneration, pancreatitis, polyamine oxidase, spermidine/spermine N^1-acetyltransferase, stereospecificity, transgenic.

1. INTRODUCTION

The natural polyamines spermidine and spermine and their precursor putrescine are aliphatic oligoamines (see the structures in Fig. (**1**)), which, due to their positive charge at physiological pH values, interact with negatively charged macromolecules and cellular components. Even though their exact physiological functions have remained elusive, a continuous supply of the polyamines is required for animal cell growth to occur. However, as an excess of the polyamines likewise is detrimental for the cells, a sophisticated system exists to maintain polyamine homeostasis. The key biosynthetic and catabolic enzymes are regulated at many levels of gene expression and also the influx and efflux of the polyamines is strictly controlled. The present review intends to highlight the metabolism and putative functions of the natural polyamines in general and those of the individual polyamines in particular with the aid of their metabolically stable α-methylated analogues. Enzymic degradation of the latter compounds has also uncovered hidden stereospecificity of polyamine oxidase, an enzyme centrally involved in the catabolism of the natural polyamines. The latter enzyme appears to be unique in the sense that the stereospecificity can be altered in the presence of certain aldehydes.

2. METABOLISM OF THE POLYAMINES

The primary precursor of putrescine, spermidine and spermine are the amino acids *L*-ornithine and *L*-methionine as shown in Fig. (**1**). Ornithine is decarboxylated by ornithine decarboxylase (ODC), a cytosolic, highly inducible enzyme with very short half-life [1, 2] to yield putrescine. The aminopropyl moieties of spermidine and spermine are originally derived from methionine, which is first activated to *S*-adenosylmethionine (AdoMet) and then decarboxylated by a second cytosolic decarboxylase, AdoMet decarboxylase (AdoMetDC). Decarboxylated AdoMet (dcAdoMet) finally donates the aminopropyl fragment to either putrescine to yield spermidine or to spermidine to yield spermine. The aminopropyl transfer reactions are catalyzed by two distinct enzymes, spermidine and spermine synthases. As the biosynthetic reactions are irreversible, an entirely different set of enzymes catalyzes the oxidative degradation of spermidine and spermine ultimately to putrescine (Fig. (**1**)). Spermidine and spermine are first *N*-acetylated by a cytosolic enzyme, spermidine/spermine N^1-acetyltransferase (SSAT) and the acetylated polyamines then serve as substrates for a peroxisomal FAD-dependent polyamine oxidase (PAO) [3]. This chain of reactions is also called polyamine backconversion pathway. Spermine, but not spermidine, can also be degraded by a recently discovered enzyme, spermine oxidase (SMO) [4, 5]. In addition to the lower polyamine, both PAO and SMO reactions generate hydrogen peroxide and aldehydes.

Fig. (**1**) (dotted arrows) likewise shows an important "side-track" of spermidine catabolism. Spermidine, but not spermine, serves as a precursor for hypusine [*N′*-(4-amino-2-hydroxybutyl)lysine]. This unusual amino acid, derived from the aminobutyl moiety of spermidine, is an integral part of functional eukaryotic initiation factor 5 A (eIF5A) [6]. As seen later, the fact that eIF5A is essential for cell proliferation [7] makes it sometimes difficult to judge whether inhibition of cell growth is primarily caused by spermidine depletion or whether it is secondary to hypusine deprivation.

3. POSSIBLE CELLULAR FUNCTIONS OF THE POLYAMINES

As indicated in the beginning of this review, a continuous supply of the polyamines appears to be an absolute prerequi-

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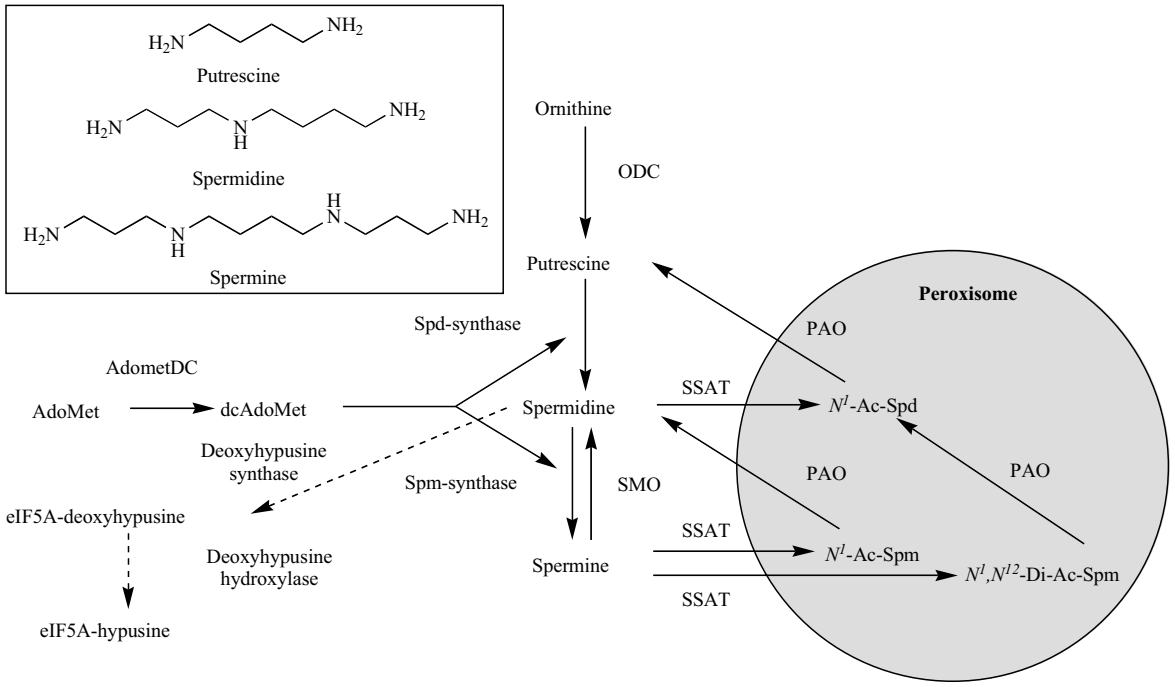


Fig. (1). Structures and metabolism of the polyamines. ODC, ornithine decarboxylase; Spd, spermidine; Spm, spermine; AdoMetDC, *S*-adenosylmethionine decarboxylase; dcAdoMet, decarboxylated AdoMet; SSAT, spermidine/spermine N^1-acetyltransferase; PAO, polyamine oxidase; SMO, spermine oxidase; eIF5A, eukaryotic initiation factor 5 A.

site for animal cell proliferation to occur. There are a vast number of reports convincingly showing that a specific inhibition of polyamine biosynthesis [8] or an activation their catabolism [9], both resulting in the depletion of cellular polyamines, will lead to cytostasis of animal cells. However, apart of the specific role of spermidine as the sole precursor of hypusine, which is required for the functional eIF5A, very little is known about the exact roles of the polyamines in growth processes. Due to their polycationic structure, the polyamines are expected to interact with negatively charged cellular molecules, such as nucleic acids and phospholipids, even with a reasonable specificity. In fact, spermidine and spermine appear to promote the conversion of right-handed B-DNA to left-handed Z-DNA and hence apparently alter the template properties of DNA [10]. There are two examples representing an extremely specific interaction between the polyamines and polynucleotides. Firstly, polyamines promote the ribosomal frameshifting in decoding ODC antizyme [11, 12], a protein inhibiting ODC activity and facilitating its degradation in the 26S proteasome [13]. Secondly, polyamine depletion appears to induce unproductive splicing of SSAT pre-mRNA, thus slowing down polyamine catabolism, whereas an excess of the polyamines or their analogues facilitate productive splicing, hence enhancing their catabolism [14].

Polyamine metabolism has been a target for cancer chemotherapy for several decades. Cellular polyamine depletion can be achieved either through an inhibition of their biosynthesis or activation of their catabolism and it almost invariably leads to growth inhibition of tumor cells. However, those impressive results obtained under cell culture conditions or even with tumor-bearing animal models, have been disappointedly poorly translated into clinical conditions. The latter failure is apparently attributable to the highly sophisticated compensatory mechanisms triggered by cellular polyamine depletion, including enhanced transport of extracellular polyamines. In contrast to human cancer studies, α-difluoromethylornithine (DFMO), a suicide inhibitor of ODC, which was synthesized already in 1978 [15], appears to be a "wonder drug" in the treatment of African sleeping sickness caused by trypanosome [10]. Unfortunately, the drug has never gained any wide-ranging use in the developmental countries due to its prohibitively high price.

4. α-METHYLATED POLYAMINES: CHEMISTRY AND SYNTHESIS

The structures of most commonly used α-methylated spermidine and spermine analogues are depicted in Fig. (**2**): compound **1**, α-methylspermidine (Me-Spd); compound **2**, α-methylspermine (Me-Spm); compound **3**, α,ω-bis-methylspermine (Me_2-Spm); compound **4**, *N*-acetyl-methylspermidine (*N*-Ac-Me-Spd). As indicated in the figure, the α-methylation does not affect the charge distribution of the natural polyamines, as the primary amino groups remain (except in compound **4**). The methylation, however, prevents the *N*-acetylation by SSAT, the rate-controlling step in polyamine degradation, rendering them metabolically much more stable than the natural polyamines.

Synthetic chemistry of polyamines and some of their analogues consisting in several methods to elongate polyamine backbone has been reviewed recently [16]. Thus, the focus in this section is to describe some of the approaches

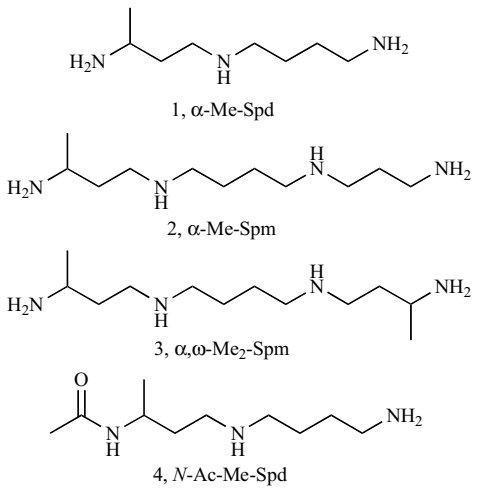


Fig. (2). α-Methylated polyamines.

used to prepare α-methylated polyamine analogues. Building blocks of α-methylated polyamines have been prepared either from masked amino functionalities (azide, N_3, or phthalimide, PhtN) or by protection of the amino group to non-nucleophilic amide derivative (carboxybenzyl, Cbz or nosyl, Ns, o-nitrophenylsulfonyl). The first synthetic strategies to prepare racemic **1**-**3**, reported by Lakanen *et al*. [17] (Scheme **1**), were not very effective, since the total yield of the end products calculated from starting materials **5** or **6** were only 7.5%, 1.7% and 17.9% (route **5**-**7**-**11**-**3**) or 5.6% (route **6**-**8**-**12**-**3**), respectively.

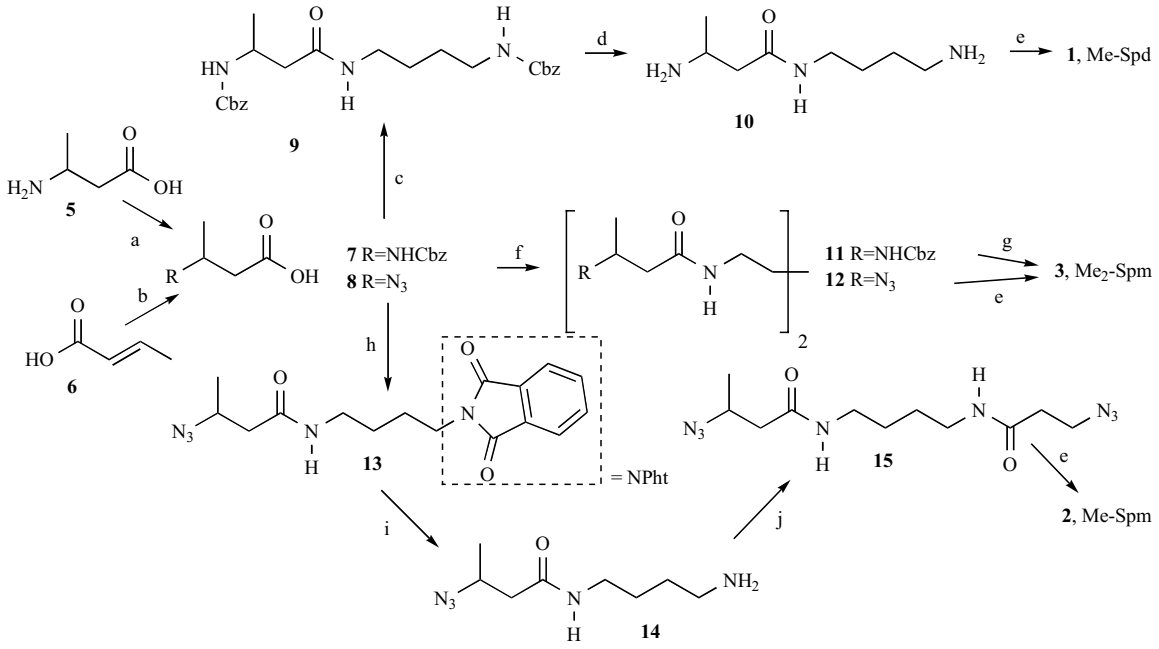
Another method to prepare racemic **1**-**3** is shown in Scheme (**2**). The key intermediate in this strategy is **19** which was synthesized from commercially available ethyl 3-aminobutyrate. The mesyl group in **19** is a good leaving group and elongation of polyamine backbone *via* this intermediate according to yields was effective, since the end product **1**-**3** were prepared with 46%, 30%, 41% calculated for starting **16**.

The first synthesis strategy to prepare optically active (*R*)- and (*S*)-α-methylated spermidines (**1**) were reported by Grigorenko *et al*. [18] and their acetylated derivatives **4** by Järvinen *et al*. [19]. Synthesis was started from enantiomerically pure cyanide **29**, which was prepared from *L*- or *D*-alaninol followed by protection and elongation of polyamine backbone to compounds (*R*)- and (*S*)-**31**. (*R*)- and (*S*)-enantiomers of **1** were obtained after deprotection with 73% and 80%, respectively, yields and corresponding acetylated derivatives with 51% and 52%, respectively, yields calculated for starting **29**. Also Lebreton *et al*. [20] have prepared several both racemic and enantiomerically pure protected spermidine derivatives, but these compounds have been used as subunits of new 15-deoxyspergualin derivatives.

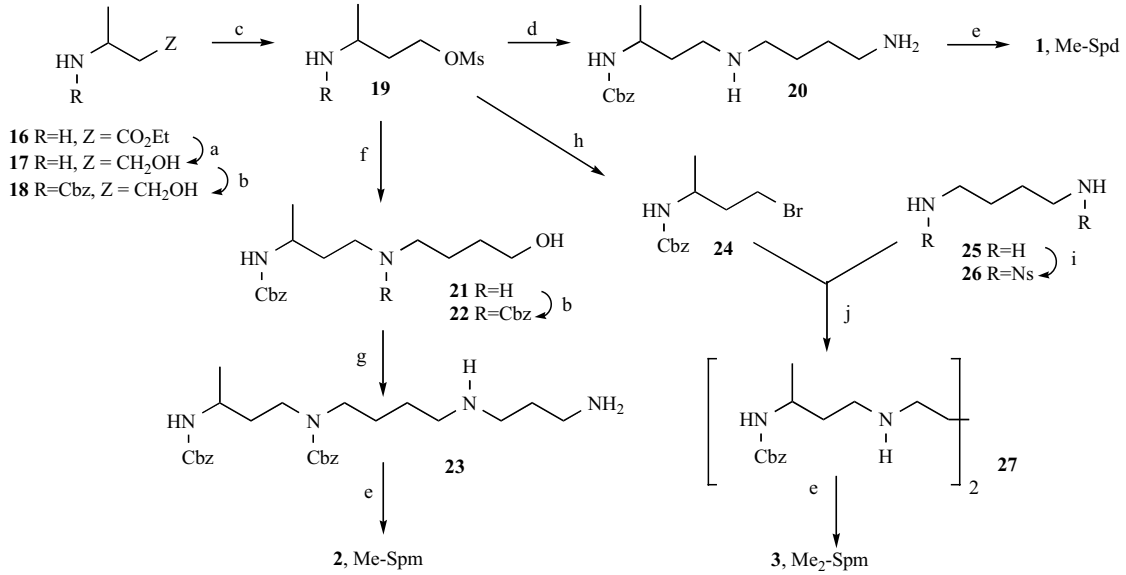
5. α-METHYLATED POLYAMINES AS SURROGATES FOR THE NATURALLY OCCURRING POLYAMINES

5.1. Studies *In Vitro*

As α-methylation of the polyamines does not affect their charge distribution and retains the terminal primary amino groups intact, it is anticipated that these methylated analogues will fulfill many of the cellular functions of the natural polyamines. In fact, many reports in the literature support this view. Methylated derivatives of spermidine and spermine have been used as substitutes for the natural poly-



Scheme (1). Reagents and conditions: a) $PhCH_2O_2CCl$; b) NaN_3, H_2O; c) $CbzNH(CH_2)_4NH_2$, DCC, HOBt; d) H_2/Pd, MeOH; e) BH_3, THF; Dowex-H^+ (two steps); f) $[H_2N(CH_2)_2]_2$, DCC, HOBt; g) H_2/PtO_2, MeOH; BH_3, THF; Dowex-H^+ (three steps); h) $H_2N(CH_2)_4NPht$, DCC, HOBt; i) N_2H_4; j) $HO_2C(CH_2)_2N_3$, DCC, HOBt.



Scheme (2). Reagents and conditions: a) $LiAlH_4$, THF, reflux; b) Cbz-Cl, H_2O, $NaHCO_3$; c) MsCl, Et_3N, 0°C; d) $H_2N(CH_2)_4NH_2$, THF, 0°C; e) H_2/Pd, AcOH-MeOH 1:1; HCl, MeOH (two steps); f) $H_2N(CH_2)_4OH$, THF, 0°-37°C; g) MsCl, Et_3N; $H_2N(CH_2)_3NH_2$, THF, 0°-20°C (two steps); h) LiBr, THF; i) NsCl, Et_3N, CH_2Cl_2, 0°C; j) K_2CO_3, DMF; PhSH, K_2CO_3, DMF (two steps).

amines both *in vitro* and *in vivo*. Circular dichroism studies with oligodeoxynucleotides have revealed that Me-Spd, Me-Spm and Me_2-Spm equally effectively induce the conversion of right-handed B-DNA to left-handed Z-DNA as do their natural counterparts [17, 21]. Moreover, polyamine depletion-associated cytostasis resulting from the inhibition of adenosylmethionine decarboxylase in cultured cells can be reversed by Me-Spd but apparently not by Me_2-Spm [22]. The lack of effect of the latter compound was thought to be attributable to a depletion of hypusine, for which Me-Spd, but not Me_2-Spm, can serve as the precursor [22]. However, Me-Spd, Me-Spm and also Me_2-Spm have been reported to reverse difluoromethylornithine-induced cytostasis in cultured cells [17]. As indicated earlier, methylated polyamines are metabolically more stable than their natural counterparts. Me-Spd is a poor substrate for spermine synthase thus being only slowly converted to Me-Spm. Me-Spd is not *N*-acetylated by SSAT and Me_2-Spm has not been reported to be metabolized at all [17]. However, we found that both the α-methylated derivatives of spermine, and to lesser extent that of spermidine, are good substrates for recombinant PAO, especially in the presence of benzaldehyde [23]. The finding that the latter compound is able to modify PAO reaction extends back to the discovery of the enzyme. Although acetylated spermidine and spermine are strongly favored substrates for PAO [24, 25], Hölttä [3] found that also unmodified polyamines were effectively degraded in the presence of certain aldehydes, especially benzaldehyde. The latter compound was thought to form Schiff base with the primary amino groups of the polyamines thus mimicking the charge distribution of acetylated polyamines. We also found that Me_2-Spm is converted to some extent to Me-Spd *in vivo* in rat liver [23] hence making Me_2-Spm as a potential precursor of hypusine. However, our subsequent experiments with cultured cells, under conditions where the conversion of Me_2-Spm to Me-Spd was totally blocked, revealed that Me_2-Spm completely reversed DFMO-induced cytostasis. These results can be understood in terms that the drug-induced early cytostasis was not related to hypusine depletion and that spermidine and spermine are fully exchangeable in supporting cell growth [23] Similar conclusions can be drawn from other experiments indicating that polyamine depletion-associated early growth inhibition occurs in the absence of a depletion of hypusinated eIF5A [26].

5.2. Studies *In Vivo*

α-Methylated polyamines have also been used to substitute the naturally occurring polyamines in a few transgenic animal models *in vivo*. We generated a transgenic rat line overexpressing SSAT under the heavy metal-inducible mouse metallothionein I promoter. Treatment of these animals with non-toxic doses of zinc, led to a striking induction of SSAT in the pancreas, profound depletion of pancreatic spermidine and spermine pools and rapid development of fulminant necrotizing pancreatitis [27]. Interestingly, it has been long known that the concentration of spermidine in the pancreas is the highest in mammalian body as is the molar ratio of spermidine to spermine [28]. As the acetylated polyamines are rapidly degraded by PAO generating hydrogen peroxide and reactive aldehydes as by-products, a plausible cause of the inflammation could have been reactive oxygen species. However, inhibition of PAO with MDL72527 [N^1,N^4-bis (2,3-butadienyl)-1,4-butanediamine], if anything, worsened the outcome of the disease [27]. The view that a depletion of pancreatic spermidine and spermine was causally related to the development of pancreatitis was strongly supported by the finding indicating that a prior administration of Me-Spd totally prevented the disease as judged by histopathology and plasma α-amylase activity [29]. Me-Spd and Me_2-Spm given after the induction of pancreatitis not only markedly alleviated the disease condition but dramatically rescued the transgenic animals from acute mortality. In fact, all the rats not

receiving the analogues died by day 3 after the zinc induction, whereas 100 % of those animals treated with Me_2-Spm and 90 % of those treated with Me-Spd were still alive 14 days after the induction of the disease [30]. Some of the experimental findings indicated that the analogues prevented the multi organ failure, which is the major cause of death in acute pancreatitis. Induction of SSAT in pancreatic acinar cells isolated from the transgenic rats led to enhanced trypsinogen activation, which, however, was preventable by a prior exposure of the cells to Me_2-Spm [30]. Interestingly, an activation of pancreatic polyamine catabolism leading to partial depletion of spermidine and spermine appears to be associated with the development of pancreatitis also in other animal models of this disease and analyses of human samples, admittedly only a few, revealed distinct polyamine depletion in acute and chronic secondary pancreatitis [30].

When the transgenic rats harboring the metallothionein promoter-driven SSAT were subjected to partial hepatectomy, SSAT was strikingly induced and the hepatic pools of the higher polyamines profoundly depleted at 24 h after partial hepatectomy. Under this condition, the transgenic animals failed to initiate liver regeneration as judged by liver weight gain, rate of DNA synthesis and number of hepatocytes positive for proliferating cell nuclear antigen (PCNA) [31]. At 3 days after the operation, when spermidine content had returned to the preoperative level (mainly due to compensatorily enhanced ODC activity), the regenerative process was restored. During the early days of regeneration, a close and highly significant correlation existed between hepatic spermidine (and spermine) content and the number of PCNA-positive hepatocytes, whereas putrescine showed negative correlation [31]. Administration of Me-Spd prior to partial hepatectomy fully restored early liver regeneration in the transgenic rats [29]. The kinetics of polyamine accumulation during liver regeneration involve an early rise of putrescine concentration followed by a marked expansion of the hepatic spermidine pool, whereas spermine content initially decreases [32]. The described kinetics may give the impression that spermidine is the most important polyamine supporting the regenerative growth, putrescine being its precursor and spermine sort of reserve pool to be converted back to spermidine. In fact, we addressed this question by giving Me_2-Spm to the transgenic rats prior to partial hepatectomy. As in case Me-Spd, the spermine analogue fully restored liver regeneration under conditions where its conversion to Me-Spd was not sufficient to support the regeneration [23]. The latter result further suggests that spermidine and spermine are fully exchangeable in supporting animal cell growth.

Taking into consideration the life-threatening nature of acute human pancreatitis and the lack of any specific treatment for it, metabolically stable polyamine analogues may offer an entirely new approach to treat this severe disease, as under the condition of activated polyamine catabolism the natural polyamines are rapidly degraded and can not be used to replenish their reduced tissue pools [33]. Similarly, these compounds may support liver integrity in case of hepatic failure.

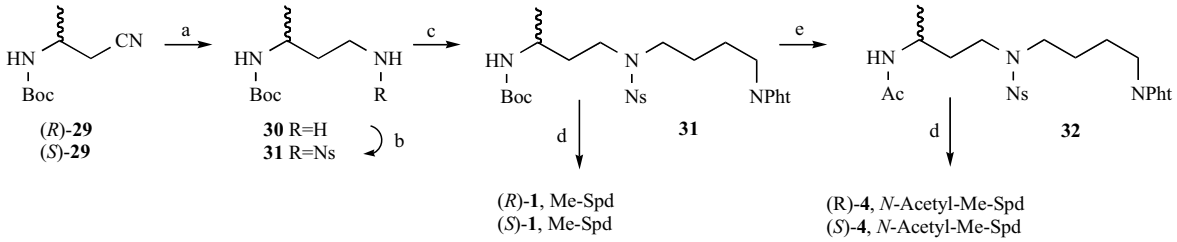
We have carried out small-scale pharmacokinetic and tolerability studies with the methylated derivatives of spermidine and spermine using nontransgenic and transgenic rats and mice. All the analogues accumulate dose-dependently in the selected tissues (liver, pancreas and kidney) studied. Their metabolism varies depending on the tissue, spermine derivatives being stable in the pancreas. The general tolerability of the analogues was good and they did not affect plasma α-amylase or ALAT activities. The LD_{50} values were fully comparable to those of the natural polyamines (intraperitoneal LD_{50} value of 870 mg/kg for spermidine and 370 mg/kg for spermine) [19].

6. ENZYMIC DEGRADATION OF A METHYLATED SPERMIDINE ANALOGUE REVEALS CRYPTIC STEREOSPECIFICITY OF POLYAMINE OXIDASE

As indicated earlier, the natural substrates for PAO are *N*-acetylated spermidine and spermine, yet in the presence of certain aldehydes also unmodified polyamines are effectively oxidized. All these substrates are achiral. We synthesized previously unknown *N*-acetyl-α-methylspermidine (1-amino-8-acetamido-5-azanonane dihydrochloride; *N*-Ac-Me-Spd; compound **4** in Fig. (**2**) as a racemic mixture and in the form of pure enantiomers (Scheme (**3**)) [19]. Using recombinant PAO, we compared N^1-Ac-Spd, the natural substrate for the enzyme, and isomers of *N*-Ac-Me-Spd as the substrates for the enzyme. Unexpectedly, PAO displayed distinct stereospecificity for the isomers of *N*-Ac-Me-Spd by strongly favoring the (*R*)-isomer as shown in Fig. (**3**). As indicated in the figure, α-methylation of acetylspermidine resulted in about 7-or 12-fold increase in the K_m value depending on the isomer. However, the k_{cat} ($V_{max}/[E]$) value for the preferred (*R*)-isomer was similar to that for the natural substrate and much higher in comparison with the (*S*)-isomer (Fig. (**3**)) [19,34]. The degradation of racemic *N*-Ac-Me-Spd was strongly influenced by the presence of the (*S*)-isomer (K_m = 100 μM; k_{cat} = 1.3 s^{-1}) [19]. It thus appears that PAO, normally splitting achiral molecules, possesses a hidden potency for stereospecificity. As α-methylated polyamines are not naturally occurring compounds, not at least in mammalian tissues, it is not excluded that PAO has some natural chiral substrate(s) not yet identified. On the other hand, the apparent stereospecificity of the enzyme could also be attributable to the steric position of the α-methyl group in the different isomers. Evidently, the inclusion of the methyl group radically decreases the affinity of PAO for both isomers in comparison with its natural substrate N^1-Ac-Spd. Depending on the given isomer, the cleavage site of the substrate may be differentially positioned in relation to the catalytically active groups of the enzyme that is then reflected as greatly different k_{cat} values. Interestingly, spermine synthase, another enzyme of polyamine metabolism normally using achiral substrates, shows stereocontrol when unsaturated derivatives of spermidine are used as substrates [35].

7. CERTAIN ALDEHYDES GUIDE THE STEREOSPECIFICITY OF THE DEGRADATION OF α-METHYLPOLYAMINES BY POLYAMINE OXIDASE

In the connection of the initial purification and characterization of rat liver PAO in 1977, Hölttä [3] found that when the enzyme activity was measured in the presence of spermidine or spermine, the reaction velocities were enhanced by various aldehydes up to 70-fold. Benzaldehyde and anisaldehyde were the most effective. The fact that when



Scheme (3). Reagents and conditions: a) $LiAlH_4$, Et_2O, -5°C; b) NsCl, Et_3N, CH_2Cl_2, 0°C; c) $PhtN(CH_2)_4I$, K_2CO_3, DMF; d) PhSH, K_2CO_3, DMF; N_2H_4, EtOH; HCl, MeOH (three steps); e) HCl, EtOH, EtAc; AcCl, Et_3N, CH_2Cl_2, 0°C (two steps).

N^1-Ac-Spd was used as the substrate aldehydes were without effect, led him to suggest that the native substrates of PAO are acetylated polyamines and that the aldehydes form a Schiff base with the primary amino group of the polyamine mimicking acetylated polyamine [3]. Using recombinant human PAO and pure enantiomers of α-methylpolyamines [18], we screened a number of aldehydes for their stimulatory effect on the oxidation reaction. Aromatic aldehydes were clearly superior to aliphatic in enhancing PAO reaction. Most of the aldehydes showed strong preference towards (*R*)-isomer of the α-methylpolyamines, benzaldehyde being the most effective one. All tested structurally related ketones were ineffective. In fact, pyridoxal was the only aldehyde, in the presence of which the (*S*)-isomer of Me-Spd was preferentially degraded, whereas pyridine 4-carboxaldehyde promoted the oxidation of both isomers [34]. Fig. (**4**) exemplifies the degradation of racemic Me-Spd, (*R*)-isomer and (*S*)-isomer of Me-Spd by rat liver extract in the presence of either benzaldehyde or pyridoxal. As indicated, in the presence of benzaldehyde PAO strongly prefers the (*R*)-isomer, whereas in the presence of pyridoxal, the (*S*)-isomer is preferred. Benzaldehyde enhanced the reaction rates of both isomers of Me-Spm and all diastereomers of Me_2-Spm, whereas pyridoxal only enhanced the oxidation of (*S,S′*)-Me_2-Spm [34].

The most likely mechanism for the aldehyde-driven enhancement of PAO activity is Schiff base formation between the aldehyde and the primary amino group(s) of the substrates mimicking the charge distribution of acetylated polyamines. This notion is indirectly supported by diverse pieces of experimental evidence. *N*-Alkylated polyamine analogues, such as N^1,N^{11}-diethylnorspermine, serve as the substrates for PAO [36]. Because of *N*-alkylation, these compounds have lost the primary amino group and cannot any more

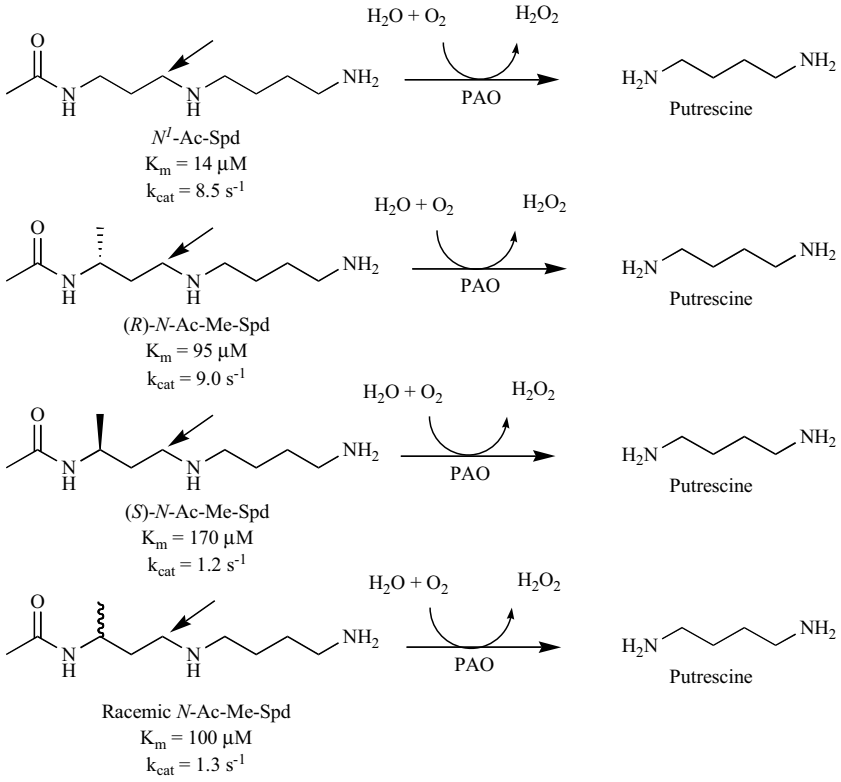


Fig. (3). Degradation of N^1-acetylspermidine and isomers of *N*-acetylmethylspermidine by polyamine oxidase (PAO).

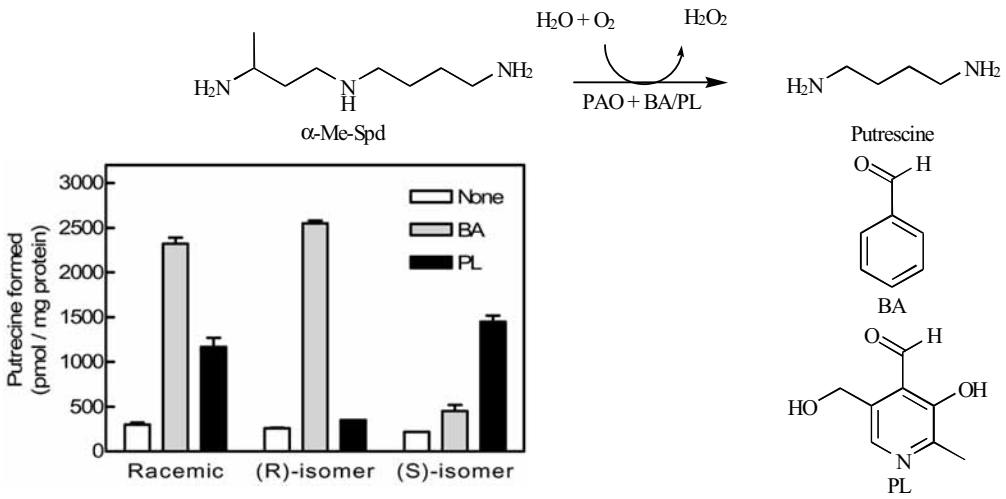


Fig. (4). Degradation of α-methylspermidine by polyamine oxidase (PAO) in the absence and presence of benzaldehyde (BA) or pyridoxal (PL). Data adapted from [34].

form a Schiff base with aldehydes. We found that using N^1,N^{11}-diethylnorspermine or N^1,N^{12}-diethylspermine as substrates in the PAO reaction, an inclusion of either benzaldehyde or pyridoxal did not enhance, but rather inhibited, the reaction rate [34]. The second piece of evidence is derived from an experiment indicating that a preformed pyridoxal=spermine adduct is a better substrate for PAO than spermine supplemented with free pyridoxal [34]. Moreover, stable mimetic of Schiff bases i.e. the oxime adducts of *N*-(aminooxyethyl)putrescine with benzaldehyde and acetone proved to be substrates of PAO. The latter finding apparently also implies that the Schiff base formation takes place in solution rather than at the active site of the enzyme and hence the Schiff base is the ultimate substrate for PAO.

The present results clearly indicate that FAD-dependent PAO, normally using achiral substrates, displays cryptic stereospecificity, and more importantly, this stereospecificity can be altered with the aid of simple aromatic aldehydes. The mechanism of the latter phenomenon is entirely unknown. There are many ways to change the stereospecificity of a given enzyme. These include covalent modification of the active site of the enzyme or modification of the substrate. An example of the former is the modification of the active site of vanillyl-alcohol oxidase with the aid of site-directed mutagenesis [37]. DFMO, a mechanism-based suicide inhibitor of mammalian ODC represents an example of substrate modification affecting the stereospecificity of an enzyme. The decarboxylation of ornithine by ODC is strictly specific to the *L*-isomer of the amino acid, yet the enzyme is equally well inactivated by *D*- and *L*-DFMO, although the affinity for *D*-isomer is about 20 times lower that that for *L*-isomer [38]. The loss of stereospecificity is thought to be attributable to the fluorine substitution of the drug [38]. Even the gender may affect the stereospecificity of an enzyme [39]. Closer to the PAO case is penicillin amidase, for which pH alters the enantioselectivity of the enzyme for charged substrates by differently ionizing the residues at the active center [40] and tryptophan synthase-catalyzed proton exchange reactions, in which the inclusion of the allosteric effector *DL*-α-glycerol-3-phosphate greatly decreases the stereospecificity of the enzyme [41].

If the observed aldehyde-guided change of the stereospecificity of PAO could be applied to other FAD-dependent or even to unrelated enzymes, this would offer a new way to generate enantiomerically pure products of an enzyme reaction, such as drugs.

CONCLUDING REMARKS

Metabolically stable α-methylated polyamine analogues have proved to be valuable experimental tools in many respects. As indicated, these compounds can be used as surrogates of the natural polyamines spermidine and spermine. The fact that they are not acetylated and are otherwise metabolized only to limited extend, makes it possible to replace the natural polyamines with them under conditions of extensive activation of polyamine catabolism, such as overexpression of SSAT. As the methylated analogues of spermidine and spermine apparently fulfill many of the cellular functions of the natural polyamines, most notably supporting animal cell growth under conditions of profound depletion of spermidine and/or spermine, they offer excellent tools to assess the cellular role of individual polyamines. As it now appears, spermidine and spermine are exchangeable in supporting growth of animal cells both *in vitro* and *in vivo*. The use of these analogues has likewise revealed that polyamine depletion-induced early growth inhibition can be dissociated from that caused by depletion of hypusinated eIF5A. However, the fact remains that spermidine, but not spermine, is the sole precursor of hypusine, the depletion of which leads to growth inhibition of eukaryotic cells [42]. It has recently been shown that *Saccharomyces cerevisiae* cells lacking spermidine synthase and FMS1-encoded amine oxidase (oxidizes spermine to spermidine like the mammalian SMO) showed absolute requirement for spermidine, not replaceable by spermine, as in the absence of the amine oxidase spermine is not converted to spermidine needed for the hypusine modification of eIF5A [43]. It is tempting to speculate that the recently discovered mammalian SMO [4, 5], specifically cleaving

spermine to spermidine, has evolved to secure a steady supply of spermidine from spermine and to ensure the formation of hypusine.

The use of the chiral α-methylated analogues of the polyamines has disclosed the cryptic stereospecificity of PAO, and even more importantly, that the stereospecificity of this enzyme can be guided by simple aromatic aldehydes. To our understanding, this is a unique phenomenon and, if applicable to other enzymes, offers entirely new possibilities for the enzymic synthesis of enantiomerically pure compounds.

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ABBREVIATIONS

AdoMet	=	*S*-Adenosylmethionine
AdoMetDC	=	*S*-Adenosylmethionine decarboxylase
dcAdoMet	=	Decarboxylated *S*-adenosylmethionine
DFMO	=	2-Difluoromethylornithine
eIF5A	=	Eukaryotic initiation factor 5 A
Me-Spd	=	α-Methylspermidine
Me-Spm	=	α-Methylspermine
Me_2-Spm	=	α,ω-Bis-methylspermine
N^1-Ac-Spd	=	N^1-Acetylspermidine
N-Ac-Me-Spd	=	*N*-Acetylmethylspermidine (1-amino-8-acetamido-5-azanonane)
ODC	=	Ornithine decarboxylase
PAO	=	Polyamine oxidase
PCNA	=	Proliferating cell nuclear antigen
SMO	=	Spermine oxidase
Spd	=	Spermidine
Spm	=	Spermine
SSAT	=	Spermidine/spermine N^1-acetyltransferase

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