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# Aminopropyltransferases: Function, Structure and Genetics

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**Aminopropyltransferases use decarboxylated *S*-adenosylmethionine as an aminopropyl donor and an amine acceptor to form polyamines. This review covers their structure, mechanism of action, inhibition, regulation and function. The best known aminopropyltransferases are spermidine synthase and spermine synthase but other members of this family including an $N^1$-aminopropylagmatine synthase have been characterized. Spermidine synthase is an essential gene in eukaryotes and is very widely distributed. Key regions in the active site, which are very highly conserved, were identified by structural studies with spermidine synthase from *Thermotoga maritima* bound to *S*-adenosyl-1,8-diamino-3-thiooctane, a multisubstrate analog inhibitor. A general mechanism for catalysis by aminopropyltransferases can be proposed based on these studies. Spermine synthase is less widely distributed and is not essential for growth in yeast. However, Gy mice lacking spermine synthase have multiple symptoms including a profound growth retardation, sterility, deafness, neurological abnormalities and a propensity to sudden death, which can all be prevented by transgenic expression of spermine synthase. A large reduction in spermine synthase in human males due to a splice site variant causes Snyder-Robinson syndrome with mental retardation, hypotonia and skeletal abnormalities.**

**Key words: aminopropyltransferase, polyamines, spermidine, spermine, *S*-adenosylmethionine, thermophiles.**

## Occurrence and role of aminopropyltransferases

Aminopropyltransferases are a very widely distributed class of enzymes that use decarboxylated *S*-adenosylmethionine (dcAdoMet) as a substrate and transfer its aminopropyl group to an amino acceptor forming an amine product with an additional positive charge and 5′-deoxy,5′-methylthioadenosine (MTA). Aminopropyltransferases are therefore responsible for the synthesis of polyamines such as spermidine and spermine. The dcAdoMet for these reactions is formed from *S*-adenosylmethionine (AdoMet) by the action of a decarboxylase (AdoMetDC). Once decarboxylated, AdoMet is not available for methyl transfer since methyltransferases do not use dcAdoMet as a substrate. Conversely, aminopropyltransferases will not use AdoMet to transfer a 3-amino, 3-carboxypropyl- group although an enzyme that does bring about this reaction to modify tRNA is known (*1*).

In this brief review, recent work on the function, structure and genetics of aminopropyltransferases will be reviewed. Only selected citations, predominantly those published in the last 5 years, are included. References to other publications and earlier papers can be found in these articles and in previous reviews (*2*, *3*).

Until recently the best characterized aminopropyltransferases were those from *E. coli*, mammals, plants and yeast. *Escherichia coli* contains only one aminopropyltransferase that has a very high degree of specificity for putrescine as the amine acceptor and forms spermidine. It is therefore frequently termed spermidine synthase (SpdS). This is consistent with the polyamine content of *E. coli* and many other bacteria that contain only putrescine and spermidine. Yeast and mammalian cells contain two aminopropyltransferases, a SpdS and a spermine synthase (SpmS) that exclusively uses spermidine as the amine acceptor and forms spermine. Plants also contain both SpdS and SpmS but have multiple forms resulting from multiple genes and alternate splicing (*4*–*7*). Thus, all eukaryotes that have significant amounts of spermine have a separate aminopropyltransferase responsible for its synthesis.

Recently other aminopropyltransferases described as SpdS have been cloned and expressed from other organisms including the pathogen *Helicobacter pylori* (*8*) and parasites such as *Plasmodium falciparum* (*9*). Aminopropyltransferases from the acute thermophiles *Thermotoga maritima* (*10*) and *Thermus thermophilus* (*11*) have been studied more extensively. Thermophiles contain a wide variety of polyamines in addition to putrescine, spermidine and spermine (*12*, *13*). It is not entirely clear whether they also contain a multiplicity of aminopropyltransferases or whether some of these polyamines are produced *via* an enzyme with highly reduced specificity for the amine acceptor particularly at high temperatures. The *Thermotoga maritima* enzyme, which is discussed in detail in the next section of this review preferred putrescine as a substrate and is therefore best referred to as a SpdS but had significant activity with other amines (*10*). The aminopropyltransferase from *Thermus thermophilus* was found to use agmatine rather than putrescine as a substrate revealing a novel biosynthetic pathway for

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spermidine in which the $N^1$-aminopropylagmatine produced is hydrolyzed by a ureohydrolase forming spermidine without using putrescine as an intermediate (*11*).

**Structure and function of aminopropyltransferases**

**Inhibitors.** There was been considerable interest in the production of specific inhibitors of aminopropyltransferases both as tools to investigate the mechanism of action of these enzymes and to perturb polyamine metabolism since the polyamine biosynthetic pathway is well established as a useful drug target. Very early studies established that cyclohexylamine was a moderately potent inhibitor of SpdS from *E. coli*, plants and mammals (*3*, *14*, *15*). The rationale of this inhibition is thought to be based on the ability of cyclohexylamine to occupy part of the putrescine binding site and an adjacent hydrophobic cavity in the active site. Direct evidence for this is lacking but it is consistent with models based on the known crystal SpdS structures (*15*). Detailed study of related compounds as inhibitors of mammalian SpdS showed that *trans*-4-methylcyclohexlamine ($IC_{50}$ of 1.7 μM) was 4 times more potent and 5-amino-1-pentene ($IC_{50}$ of 1.7 μM) and pentylamine ($IC_{50}$ of 3.6 μM) were also more effective (*14*, *15*). Interestingly, the *Thermotoga maritima* spermidine synthase (TmSpdS) is not inactivated by cyclohexylamine although it is weakly inhibited by pentylamine (unpublished observations).

Inhibitors of SpmS based on the same concept were made by adding hydrophobic groups to 1,3-diaminopropane (*3*, *14*). This resulted in even more potent compounds than the SpdS inhibitors. The most active compounds were *N*-(3-aminopropyl)cyclohexylamine ($IC_{50}$ of 0.2 μM) and *N*-butyl-1,3-diaminopropane ($IC_{50}$ of 0.4 μM). Aminooxy analogues of spermidine, 1-aminooxy-3-*N*-(3-aminopropyl)-aminopropane ($K_i$ 1.5 μM) and *N*-[(2-aminooxyethyl)-1,4-diaminobutane ($K_i$ 186 μM) were good competitive inhibitors and poor substrates of SpmS (*16*) but these polyamine analogs have multiple effects on polyamine metabolism.

Much more potent aminopropyltransferase inhibitors were produced by Coward and colleagues using models of the transition-state of a direct displacement mechanism to design multisubstrate inhibitors (*3*, *17*). Thus, *S*-adenosyl-1,8-diamino-3-thiooctane (AdoDATO) was a very potent and selective inactivator of SpdS from mammals and *E. coli* ($IC_{50}$ c. 50 nM) and *S*-adenosyl-1,12-diamino-3-thio-9-azadodecane (AdoDATAD) ($IC_{50}$ c. 20 nM) was effective against SpmS. An even more potent inactivator of SpdS was adenosylspermidine [3-(*RS*)-(5′-deoxy-5′-carbaadenos-6′-yl)spermidine] ($IC_{50}$ c. 14 nM) but this compound was less selective and inhibited SpmS with $IC_{50}$ of 750 nM).

Treatment of cultured cells or rats with inhibitors of SpmS leads to the expected result of a decrease in spermine and an increase in spermidine, which is frequently greater than the fall in spermine leading to an increase in total polyamines. This increase is due to a reduced level of repression of ornithine decarboxylase (ODC) and AdoMetDC. The resulting elevations in these enzymes provides increased substrates for SpdS. Little, if any effect on growth was observed in most of these studies (*3*) although *N*-(3-aminopropyl)cyclohexylamine had a significant antiproliferative effect in ZR-75-1 breast cancer cells, a line in which the accumulation of spermidine was least, suggesting that spermidine can replace spermine if present in high amounts. In rats treated with *N*-(3-aminopropyl)-cyclohexylamine there was no effect on growth or physiology but, in many tissues, spermine was not fully depleted (*18*).

Exposure of cultured cells to AdoDATO or other SpdS inhibitors lead to a fall in spermidine but to a rather paradoxical increase in spermine (*3*). Recently, similar changes in polyamines have been seen in rat tissues after treatment with *trans*-4-methylcyclohexlamine or 5-amino-1-pentene (*19*). It has been suggested that these alterations in polyamine levels are due to the increased synthesis and availability of dcAdoMet which allows SpmS to very effectively convert spermidine into spermine but this provides only a partial explanation. It is still unclear why the total of spermidine and spermine is not much reduced when SpdS is inhibited since all the higher polyamines must come through this reaction. A likely factor is that the increase in AdoMetDC and its supply of dcAdoMet is such that SpdS forms spermidine at a normal rate even though it is inhibited.

MTA, the nucleoside product of the aminopropyltransferase reactions is a moderately good inhibitor of the reactions particularly that of SpmS (*20*, *21*). This is normally unimportant in controlling polyamine synthesis since MTA is rapidly degraded by MTA phosphorylase in mammals and MTA nucleosidase in some microorganisms. However, the MTA phosphorylase gene is deleted in many tumors and potent inactivators of MTA phosphorylase are now being examined as therapeutic agents (*22*). If MTA is allowed to accumulate, inhibitory effects on the formation of aminopropyltransferase products would be expected.

**Structure.** Most aminopropyltransferases that have been characterized are homodimers although those from thermophiles are tetramers. In *Arabidopsis thaliana*, which contains at least two SpdS and two SpmS, heterodimers of the gene products from *SpdS1*, *SpdS2* and *SpmS1* were formed *in vitro* and they were present in large complexes of 650–750 kDa in cell extracts (*4*). The significance of this observation for polyamine synthesis and possible substrate channelling is not clear.

The most significant recent breakthrough in understanding aminopropyltransferases has come with the determination of a high resolution structure (1.5 Å) of the TmSpdS in the presence and absence of the multisubstrate analog inhibitor, AdoDATO (*10*). This SpdS was remarkably stable to thermal denaturation particularly in the presence of the amine acceptor substrate putrescine where the half life was >25 h at 90°C. The $k_{cat}$ value for aminopropylation of putrescine increased with temperature with a 29-fold rise from 37°C to 80°C but the $K_m$ value for putrescine increased slightly from 0.19 mM to 0.32 mM (unpublished). Several other amines including 1,3-diaminopropane, cadaverine, agmatine, *sym*-norspermidine (thermine) and spermidine could be used as acceptors of the aminopropyl group but all were worse substrates than putrescine. At 37°C, the maximal reaction with spermidine occurred at 18% of the rate with putrescine and the corresponding values for the other amines were thermine (37%), 1,3-diaminopropane (23%) and 1,5-diaminopentane (4%). At 65°C, the relative rates were spermidine (31%), thermine (15%), 1,3-diaminopropane (11%) and 1,5-diaminopentane (8%).

The TmSpdS is tetrameric. Each monomer consists of a C-terminal domain with a Rossman-like fold and an N-terminal β-stranded domain (*10*). The tetramer is maintained by a tight β-barrel with a strong hydrophobic core, which is formed by the four N-terminal β-hairpins. This is likely to be involved in the extreme thermostability of the TmSpdS since oligomerization is known to increase protein stability by reducing the solvent-accessible surface. Aminopropyltransferases from other thermophiles are also tetramers whereas SpdS from non-thermophiles are dimers. In both cases, the monomer unit contains an active site made up of residues only from that subunit. The independence of these sites suggests that the difference between the tetrameric and dimeric aminopropyltransferases does not invalidate the use of the TmSpdS structure for general modelling of the aminopropyltransferase reaction.

The active site of the TmSpdS is revealed well by the interactions with AdoDATO (Fig. 1A). This binding site is lined with residues conserved in all aminopropyltransferases suggesting a conserved catalytic mechanism (*10*). The structure reveals deep cavities for binding substrate and cofactor and a loop made up of residues 171–180 that envelopes the active site. The structure of the free and inhibitor-bound enzyme reveals a significant conformational change in this 171–180 loop upon binding of AdoDATO (Fig. 1B). Therefore, the 171–180 loop is effectively a gatekeeper to and from the active site. The polyamine moiety of AdoDATO is oriented toward a long, deep cleft between the N- and C-terminal domains. The surface of the cavity is hydrophobic in the central region with negative charge distribution at both ends, which should anchor the amino groups of putrescine. The length of this partially hydrophobic polyamine–binding cavity is likely to be a key feature for selecting the polyamine acceptor. The structure also allows the suggestion of a model for catalysis, which is described in detail below.

Several other aminopropyltransferase structures are now available including those of the human, *Caenorhabditis elegans*, *Arabidopsis thaliana*, *Bacillus subtilis*, *Pyrococcus furiosus* and *Aquifex aeolicus* SpdS and the $N^1$-aminopropylagmatine synthase from *Thermus thermophilus* although not in complexes with substrates or inhibitors. Crystallization of a putative SpdS from *Helicobacter pylori* has been reported (*23*). The latter structure would be of considerable interest since the amino acid sequence of the *Helicobacter pylori* protein is quite different from that of other SpdS in the putrescine binding region and lacks the gate keeping loop described below. However, it should be noted that the determination of the substrate specificity of this enzyme was incomplete, showing production of spermidine with 0.5 mM putrescine and 100 μg of recombinant enzyme (*8*). No other substrates except spermidine, which was not active, were tested so it is possible that this enzyme has a preference for another amine acceptor.

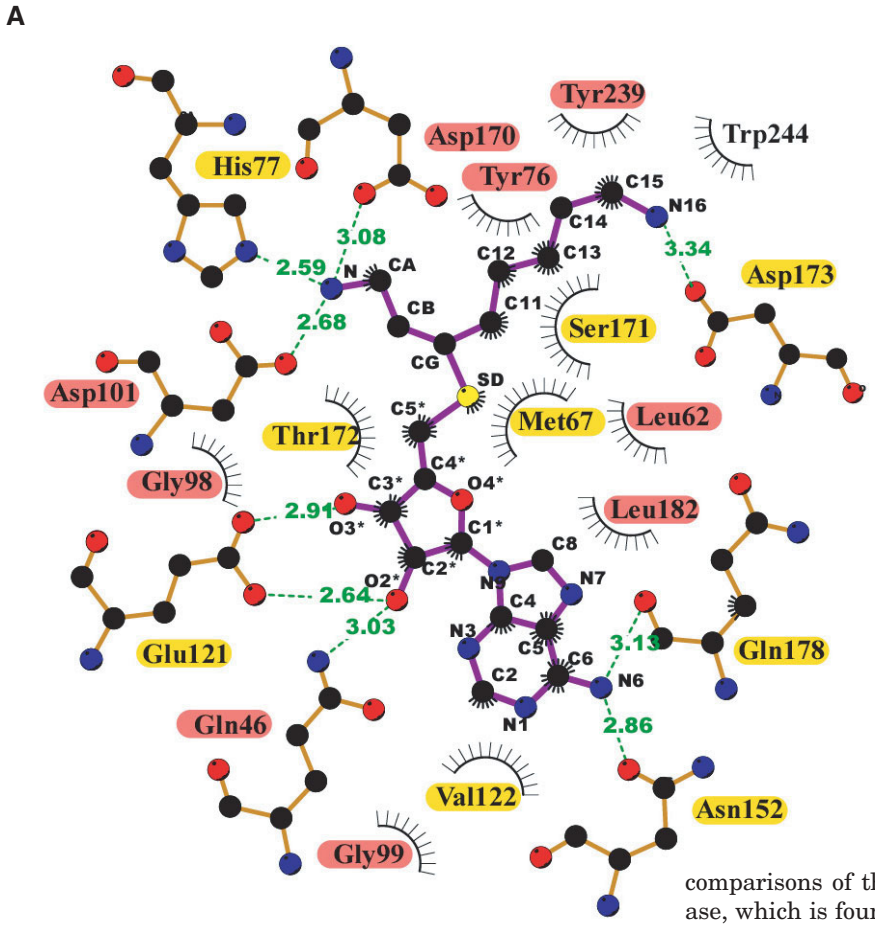
**Model for reaction of aminopropyltransferases**

A model for the aminopropyl transfer reaction is shown on the left side of Fig. 2. Formation of spermidine from putrescine is suggested to occur by a single-displacement mechanism, with inversion of the configuration of the methylene carbon undergoing nucleophilic attack by putrescine. This is in agreement with the known stereochemistry of the reaction with *E. coli* SpdS (*24*, *25*). The interaction of residues from the enzyme with the amino group of putrescine to reduce its protonation would be required to facilitate the reaction. Analysis of the structure points to Asp170, which is conserved in all aminopropyltransferases, as the most likely candidate to deprotonate the attacking amino group of putrescine. The main chain carbonyl of Ser 171 and the hydroxyl group of the side chain of Tyr76 (also fully conserved), which is on the opposite side of the catalytic cavity with the OH group pointing towards $C_{11}$ of AdoDATO in the inhibitor:enzyme complex, are also likely to aid in the activation, binding and proper orientation of putrescine. The distal end of the putrescine is likely to interact with conserved residue Asp173. Thus, a mechanism can be proposed in which putrescine binding involves interaction with Asp173 at the distal amino group and Asp170, Ser171 and Tyr76 at the proximal amino group allowing its attack on the $C_1$ of the propylamine group attached to the sulfonium center of dcAdoMet.

Analysis of mutants Y76F, D170A and D173A in TmSpdS is consistent with this proposal. All were all much less active than wild type with mutation D170A producing the greatest effect with >5,000-fold reduction in $k_{cat}$ (unpublished observations). The D173A mutant has a reduction in $k_{cat}$ of about 250-fold and increased the $K_m$ for putrescine by 240-fold. Similar analysis of mutants of human SpmS is also consistent with this general mechanism. Although no crystal structure is currently available for this or any other SpmS, residues equivalent to some of those described in this mechanism are easily identified from sequence comparisons. Thus, residue Asp276 in human SpmS is equivalent to Asp170 in TmSpdS and Tyr177 is equivalent to Tyr76. Mutation of these residues in human SpmS leads to a major reduction in $k_{cat}$ (unpublished observations).

**Specificity for dcAdoMet and relation to methyltransferases**

The structure of TmSpdS bound to AdoDATO provides a clear indication of the residues interacting with the adenosine nucleoside substrate (Fig. 1A). The superposition of the TmSpdS catalytic core structure with those of known AdoMet/methyltransferase complexes reveals a very similar conformation and orientation of the adenosyl moiety. The side chain of conserved residue Glu121 interacts with the ribosyl hydroxyl groups and Gln178 and Asn152 interact with the 6-amino group (*10*). The carbon of the aminopropyl moiety that is linked to the sulphur of AdoDATO is oriented approximately in the same direction as the target methyl group of AdoMet in methyltransferases. This orientation positions the target group of the aminopropyl moiety toward the putrescine binding cavity. The absolute specificity of aminopropyltransferases for dcAdoMet is explained by the invariant presence of an acidic residue at the position equivalent to Asp101 in TmSpdS and a narrower binding cavity than the analogous cavity in methyltransferases (*10*). Thus, AdoMet cannot be accommodated since its carboxyl group would undergo a steric and electrostatic clash with the side chain of Asp101, which is located in the totally conserved sequence -GGGDG-. Methyltransferases have an Ile at this position. Mutation of Asp101 in TmSpdS or the equivalent residue in human SpmS (Asp201) to Ile or Asn produce large increase in $K_m$ for dcAdoMet and a >2,000-fold reduction



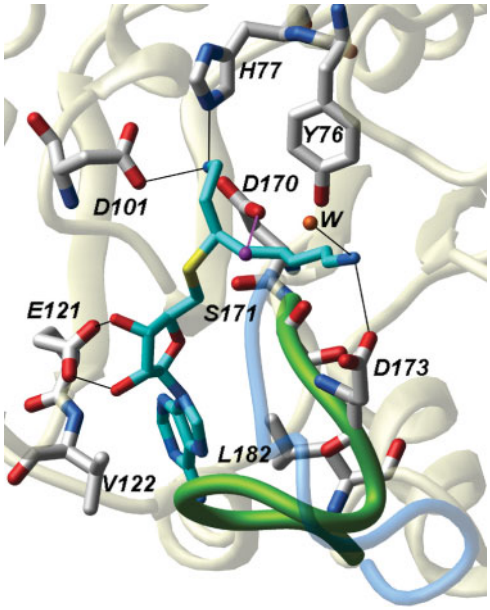




Fig. 1. **Structure of the active site of TmSpdS.** (A) A schematic diagram of the residues in AdoDATO (violet) and those in TmSpdS. Residues absolutely conserved in all aminopropyltransferases are shown in red and those on which only highly conservative replacements such as Asp for Glu are shown in yellow. Hydrogen bonds between amino acid residues and the multisubstrate inhibitor are shown as dotted green lines with distances. (B) A view of the active site in TmSpdS with AdoDATO attached and some key residues shown in the ball and stick representation. W indicates a water molecule. The key role of the side chain of Asp170 in interaction with the position that would be occupied by the attacking amino group of putrescine is shown as a magenta line The position of flexible "gate keeping" loop is shown as a green worm in the closed structure around AdoDATO, its position in the open unbound form is shown by a light blue worm. Reprinted by permission from Macmillan Publishers Ltd from *Nature Struct. Biol.* **9**, 27–31 copyright (2001) (*10*).

in $k_{cat}$ confirming the importance of this residue (unpublished observations).

The close relationship between aminopropyltransferases and methyltransferases is readily demonstrated by comparisons of the enzyme putrescine *N*-methyltransferase, which is found in some plants as part of the pathway making alkaloids. In the regions identified as part of the active site cleft in SpdS there is remarkable identity to putrescine *N*-methyltransferase with all of the residues contributing to putrescine binding and catalysis and some of those binding the nucleoside such as Glu121, are also conserved. In addition to the Asp101 change to Ile, there are only 5 other alterations in the active site region between TmSpdS and putrescine *N*-methyltransferase (Asp72 to Gly, Glu73 to Gly, His77 to Thr, Gly103 to Phe, Arg106 to Phe). These alterations presumably allow binding of AdoMet and alter the orientation of the nucleoside so that the putrescine can attack the methyl group rather than the 3-amino-3-carboxypropyl-moiety.

### Amine substrate specificity

SpdS will use cadaverine and 1,3-diaminopropane in place of putrescine but these are usually very poor substrates. Non-physiological substrates for SpdS include *N*-methylputrescine and 4-aminomethylpiperidine (*26*). When tested using large amounts of recombinant enzyme and substrate, most if not all, SpdS enzymes will use spermidine and form spermine to a small extent. but this reaction is probably physiologically insignificant occurring at less than 1% of the rate at which putrescine is used. As described above, TmSpdS is somewhat less strict in substrate requirements than human SpdS and may contribute to the production of longer chain polyamines.

The specificity of SpdS for putrescine is readily explained by (a) the positioning of Asp170 and Asp173 and other residues in that region, which form a pocket that

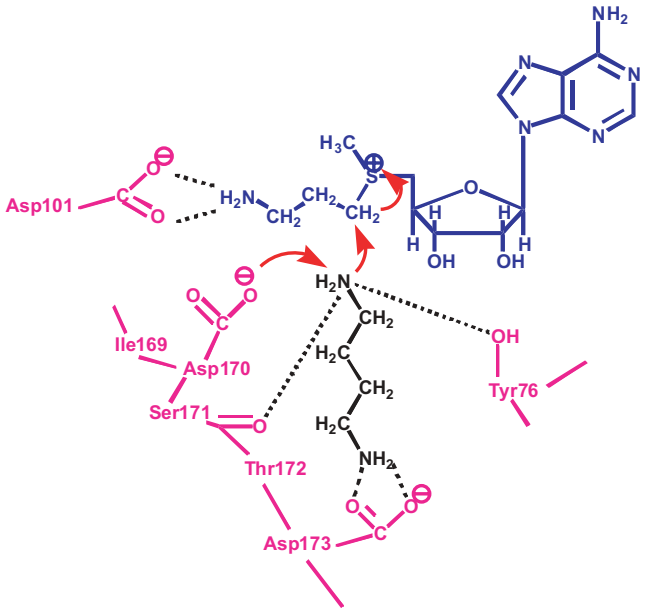



Fig. 2. **Proposed mechanism for aminopropyl transfer.** The putrescine substrate is shown in black and the dcAdoMet substrate in blue. The proposed attack by the putrescine amino group on the methylene carbon of the aminopropyl group is indicated by red arrows. This attack is facilitated by interactions of the amino group with the side chains of Tyr76 and Asp170 and the carbonyl group of Ser171. Residues Asp173, which interacts with the other amino group of putrescine, and Asp101, which interacts with the amino group on dcAdoMet are also shown. Residues in TmSpdS are shown in purple and interactions as black dotted lines.

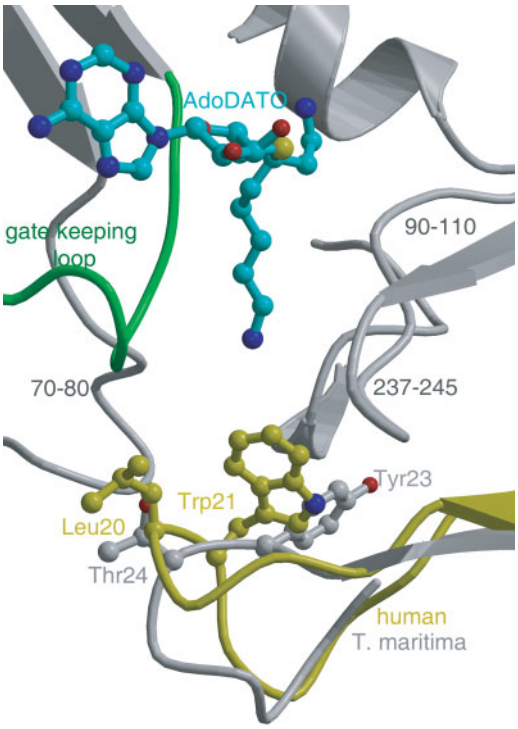



Fig. 3. **Active site of SpdS highlighting end of Spd binding cavity.** In human SpdS, shown in yellow, the cavity is closed off by Trp21. By contrast, this region in TmSpdS, shown in gray, is more open due to the position of Tyr23. Regions previously identified as binding AdoDATO are colored gray and shown in ribbon representation for clarity, with the exception of the gatekeeping loop which is colored green. AdoDATO is shown in ball-and-stick representation and colored cyan. Oxygen and nitrogen atoms are colored red and blue respectively.

recognizes putrescine much better than related diamines. The positioning of the flexible gate keeper loop that contains Asp173 is likely to impact on substrate specificity. This loop in TmSpdS contains one more residue and has one less Pro than in other SpmS proteins such as the human. This may lead to a greater flexibility and allow for lesser specificity of TmSpdS. The $N^1$-aminopropylagmatine synthase from *Thermus thermophilus* has an alteration in the orientation of the residue equivalent to Asp173 that reduces the ability to recognize putrescine and other alterations that allow agmatine to be preferred (T. Ganbe *et al.*, unpublished; PDB ID=1UIR).

The inability of SpdS to use spermidine as a substrate is probably due a steric clash that prevents the longer substrate from binding. A comparison of the available structures of SpdS shows that the loop between β-strands β1 and β2, in particular Trp21 in human SpdS contributes to the closing of the active site pocket (Fig. 3). This Trp (which is highly but not totally conserved and is residue 18 in TmSpdS) is located in a loop that is involved in oligomerization, although its conformation does not appear to correlate with oligomerization state, but rather with substrate selectivity. In the structure of human SpdS, which is very highly specific for putrescine, the Trp21 side-chain closes off the substrate binding pocket. By contrast, in the structure of the more promiscuous SpmS from *T. maritima*, the corresponding loop is one amino acid shorter and produces a larger binding cavity, which may be limited, but to a lesser extent by residue Tyr23 (Fig. 3) and also defined by the flanking residues Trp244 (which is not conserved) and conserved residue Tyr239 (Fig. 1A),

The amine substrate specificity of SpmS is not well understood but must relate to the amino acid sequence in the binding pocket. Presumably, the spermidine is positioned by interactions of the amino groups with positively charged residues in the protein and hydrophobic interactions with the spermidine aliphatic residues but the sequence divergence after position Asp276 (Asp170 in TmSpdS) is too great for useful modelling.

## Genetics and cellular physiology of aminopropyltransferases

**Inactivation of spermidine synthase.** *E. coli* can grow without spermidine but deletion of *SpdS* in *Saccharomyces cerevisiae* (*27*), *Dictyostelium discoideum* (*28*), *Leishmania donovani* (*29*), *Aspergillus nidulans* (*30*), results in strains requiring spermidine for growth. The ability to permit growth of such strains has been used to characterize gene products as SpdS (*7*, *28–31*). This conclusion certainly shows that, at the levels expressed, the protein is able to make spermidine but more detailed biochemical characterization is needed to establish that putrescine is indeed the preferred substrate. A novel aminopropyltransferase in the fungal pathogen *Cryptoccus neoformans* was identified in this way. This protein is a chimera that fuses SpdS and saccharopine dehydrogenase, an enzyme in lysine biosynthesis. The deletion of this gene imparts a requirement for both lysine and spermidine (*31*).

Deletion of one of the two *SpdS* genes in *Arabidopsis thaliana* had no effect but deletion of both significantly reduced spermidine levels and led to arrest of growth at a very early stage (*32*). Knockouts of *SpdS* in mice have not been reported but are expected to be lethal at an early stage since deletion of AdoMetDC, whose only known function is to provide the substrate for aminopropyl transfer, is lethal before embryonic day 6 (*33*).

**Inactivation of spermine synthase.** SpmS is not essential for growth of yeast since mutants lacking the *SpmS* gene grew normally (*34*) and mutants lacking the *AdoMetDC* gene, which cannot make spermidine or spermine, grow normally when only spermidine is provided (*35*). Deletion of *SpmS-1* in *Arabidopsis* reduced spermine content but had no effect on the phenotype (*36*). However, deletion of the other gene (*ACL5*) causes a severe defect in stem elongation (*6*, *36*) and a mutation in this gene has been associated with a defect causing increased vascularization and thicker veins (*37*). It is suggested that polyamines (presumably spermine) are involved in polar auxin transport, which regulates the induction of vascular tissue. The deletion of both genes still led to viable plants with no detectable spermine but with the reduced stem growth associated with loss of *ACL5*. This gene may encode a SpmS specific for provascular cells (*37*).

SpmS is clearly needed for normal mammalian development. The *SpmS* gene is located on the X chromosome in the Xp22.1 region in both humans and mice. Attempts to generate knockout mice that lack *SpmS* have not been successful probably due to the lack of viability in the 129/SvJ background. However, mice lacking *SpmS* have been identified as a result of exposure to mutagens. The male Gyro (Gy) mouse, which is on the B6C3H background, has an X chromosomal deletion, which spans 160–190 kb and includes most of *SpmS* and the adjacent *Phex* genes (*38*). The *Phex* gene product is involved in regulating phosphate metabolism and the male Gy mice carrying this deletion have symptoms of hypophosphatemic rickets. However, they also have a variety of defects including inner ear abnormalities, deafness, hyperactivity, circling behavior, sterility, a profound growth retardation and a propensity to sudden death. It is likely that these features are due to the lack of SpmS since: (a) two other mouse strains that inactivate Phex without effects on SpmS are known and these mice have hypophosphatemia but do not show these alterations; and (b) these symptoms, but not the hypophosphatemia, can be abolished in crosses of Gy with CAG/SpmS transgenic mice, which as described below, express SpmS from the ubiquitous CAG promoter and restore spermine in all tissues examined. Therefore, it appears that SpmS, and probably its spermine product, are essential for normal mammalian growth and development even though no effect on growth is seen in isolated cells in culture grown in the presence of SpmS inhibitors (see above) or in embryonic fibroblasts derived from Gy mice (*39*). Tissues from Gy mice contained no spermine but had increased levels of spermidine. It cannot be ruled out that the deleterious effects of the Gy mutation are due to over-accumulation of spermidine rather than loss of spermine but a more attractive hypothesis is that there is an essential function or functions of spermine in some cell types. At present, this function is unclear but it is very interesting that the phenotype of the Gy mice is similar to that of the mice in which the NKCC1 transporter has been knocked out (*40*). Another interesting feature is that attempts to transfer the Gy phenotype from the B6C3H background have been unsuccessful. It is possible that a modulating gene allows survival in this strain.

It should be noted that, in Gy mice, there is a substantial rise in spermidine, which is larger than the amount of spermine that is not produced. This effect, which is similar to the results seen in cells grown in the presence of SpmS inhibitors, appears to be due to a compensatory increase in other enzymes of polyamine metabolism particularly ODC and AdoMetDC. The increased supply of substrates for SpdS is likely to be the critical factor in making more spermidine since changes in SpdS in Gy mice were small and increased in some tissues such as heart but decreased in others such as brain.

A defect in human SpmS is the cause of Snyder-Robinson syndrome, an X-linked mental retardation disorder (*41*). Fibroblasts and lymphoblasts from males suffering from this condition have a greatly reduced level of SpmS. Sequence analysis of the *SpmS* gene revealed a single base substitution of G to A at position +5 of the 5′ splice site of intron 4. This change, which alters the exon 4/intron 4 boundary from CGgtaa**g**t to CGgtaa**a**t and reduces the splice consensus value, segregated with affected status. RT-PCR analysis of SpmS mRNA from affected males gave two bands on agarose gel electrophoresis. One band was of the expected size for full length SpmS mRNA, which is also seen in normal controls, and one was smaller and unique to affected individuals and carriers. Sequence analysis of the lower band from the patients revealed the absence of exon 4 while sequence analysis of the control band was consistent with proper splicing. The predicted SpmS protein from the splice variant would be missing 22 amino acids from exon 4, and contain 22 novel amino acids prior to truncation at position 111. This severe truncation would remove the entire active site of SpmS and it is impossible that the residual activity in cells arises from this protein. However, since there was some mRNA of the normal size, read-through at the splice site allows for some normal protein to be made. Such read-through may be cell dependent and could account for the observation that SpmS levels were reduced to c. 3% in lymphoblasts compared to c. 12% in fibroblasts. These changes in SpmS greatly perturbed polyamines with a substantial rise in the spermidine:spermine ratio but there was little fall in spermine. It appears that compensatory increases in synthesis of spermidine maintain the total polyamine content.

It seems very probable that the alteration in polyamine levels causes the symptoms of Snyder-Robinson syndrome, which include mild-to-moderate mental retardation, hypotonia, cerebellar circuitry dysfunction, facial asymmetry, thin habitus, osteoporosis and kyphoscoliosis. These are quite different from those in Gy mice but it is not known if this is due to a species difference, or to the fact that the Gy mice have no spermine at all. The extent to which spermine is altered in the tissues of Snyder-Robinson patients is unknown and, based on the results with cultured cells, may (a) vary according to cell type and (b) may involve only a rise in spermidine rather a major fall in spermine

**Transgenic alterations of aminopropyltransferase activity**

Transgenic overexpression of SpdS in *Arabidopsis thaliana* increased tissue spermidine content slightly (1.3 to 2-fold) and also increased spermine, which is very low in plants, by about the same amount. Remarkably, these transgenic plants showed an enhanced tolerance to stress induced by multiple stimuli (*42*). Transgenic overexpression of SpdS in tobacco has also been reported to increase spermidine levels, but not total polyamines, without deleterious effects (*43*).

Transgenic mice with a widespread increase in SpmS activity were generated using the composite CMV-IE enhancer/chicken beta-actin promoter (*44*). Very large increases in SpmS were found in all of the tissues examined but the relative SpmS activities varied widely according to the tissue studied. For example, SpmS was increased 500–2,000 fold in the heart depending on the line examined. SpdS activity was not affected. Although there was a statistically significant decline in spermidine content and increase in spermine, the alterations were small compared to the increase in SpmS. Thus, the levels of higher polyamines spermidine and spermine are not determined solely by the relative activities of the two aminopropyltransferases.

The CAG/SpmS mice had normal growth, fertility and behavior up to the age of at least 12 months. However, breeding the CAG/SpmS mice with MHC/AdoMetDC mice was lethal (*44*). The MHC/AdoMetDC mice express AdoMetDC from the very strong and heart specific α-myosin heavy chain promoter and their cardiac AdoMetDC was increased by >100 fold (*45*). These results suggest that the supply of decarboxylated AdoMet limits spermine production and prevents spermine from reaching toxic levels even when there is a >500-fold increase in SpmS.

**Regulation of aminopropyltransferases**

Polyamine metabolism is very highly regulated to maintain polyamine homeostasis and to modulate polyamine levels according to cell physiology. Many fold alterations in the levels of key enzymes such as ODC, AdoMetDC and spermidine/spermine $N^1$-acetyltransferase occur in response to physiological and pathological stimuli. Reports of alterations in aminopropyltransferase levels are much less common and it is generally accepted that the formation of the products of these enzymes is determined by the availability of the substrates. The studies with CAG/SpmS transgenic mice and cells from Snyder-Robinson patients described above also indicate that SpmS levels can vary widely without effect on spermine content due to compensatory mechanisms. However, there are a number of reports describing alterations in SpdS activity and it is possible that such changes have been overlooked because studies have focussed on the decarboxylases that are easier to assay than the aminopropyltransferases. For example, we have recently observed a small but statistically significant increase in SpmS in the hearts of MHC/AdoMetDC mice which have a >100 fold increase in AdoMetDC (*45*). SpmS has also been shown to be down-regulated in response to TGF-β and increased during liver regeneration, hormonal stimulation and lectin-induced lymphocyte activation [(*46*, *47*) and references therein].

**Aminopropyltransferases and regulation of polyamine content**

The results of the studies described above indicate that the relative levels of higher polyamines are not solely determined by the activities of SpdS and SpmS as measured in *in vitro* assays. Multiple compensatory mechanisms are likely to maintain spermine and spermine content. These include rises in ODC and AdoMetDC when polyamine levels, particularly spermine, fall and an increased degradation via the acetylpolyamine oxidase: spermidine/spermine-$N^1$-acetyltransferase or possibly the spermine oxidase pathways (*48*) when polyamine levels rise. It is therefore possible that when SpmS is increased a futile cycle occurs in which extra spermine is made and then degraded. This would use up AdoMet but the capacity to form this nucleoside and its decarboxylated derivative may be expanded as necessary to maintain polyamine homeostasis. Similarly, when SpmS is decreased, as in Snyder-Robinson syndrome, an increased production of dcAdoMet and putrescine by increased decarboxylase activity would cause a rise in spermidine levels. This increase in spermidine and the increased dcAdoMet content may allow spermine to be maintained even though SpmS is low. Other possibilities include compartmentalization of the enzymes and substrates or the possibility that complexes between the aminopropyltransferases such as those reported in plants may influence the disposition of the available dcAdoMet. Experimental proof of these concepts, which depend on the assumption that the formation of aminopropyltransferase products is limited by availability of substrates, requires measurement of flux through these enzymes using tracers and detailed studies on the subcellular localization of aminopropyltransferases and any interacting proteins.

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