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Enigmas of Polyamine Metabolisms of *Thermus thermophilus* HB8: Is sym-Homospermidine Synthesized by a Unique Enzyme?

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Novel Polyamines

Thermus thermophilus produces many unique polyamines, especially long polyamines such as caldopentamine and caldohexamine (for chemical structures, see Table 1) and branched polyamines such as tetrakis(3-aminoproyl)ammonium. The following experimental results indicated that these unique polyamines are essential for life at high temperature extremes of *T. thermophilus*;

1 The unique polyamines are abundant in the cells grown at higher temperatures such as 75°C[1].

2 Knock-out mutants which cannot produce the unique polyamines cannot grow at 75°C or higher temperatures and the addition of the unique polyamines restored the growth at 75°C (details unpublished).

3 *In vitro*, nucleic acids (DNA and RNA) are more efficiently stabilized by the addition of the unique polyamines[2].

4 *In vitro* protein synthesis at 65°C or higher temperature catalyzed by a cell-free extract of *T. thermophilus* requires absolutely the addition of polyamines and the highest activity was observed when a branched polyamine, tetrakis(3-aminopropyl)ammonium, was added to the reaction mixture[3].

Table 1 Polyamines found in *T. thermophilus* HB8.

Systematic name (Trivial Name)	Chemical structure
1,3-Diaminopropane	$NH_2(CH_2)_3NH_2$
1,4-Diaminobutane (Putrescine)	$NH_2(CH_2)_4NH_2$
1,7-Diamino-4-azaheptane (Norspermidine, Caldine)	$NH_2(CH_2)_3NH(CH_2)_3NH_2$
1,8-Diamino-4-azaoctane (Spermidine)	$NH_2(CH_2)_3NH(CH_2)_4NH_2$
1,9-Diamino-5-azanonane (*sym*-Homospermidine)	$NH_2(CH_2)_4NH(CH_2)_4NH_2$

1,11-Diamino-4,8-diazaundecane (Thermine)	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$
1,12-Diamino-4,9-diazadodecane (Spermine)	$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$
1,12-Diamino-4,8-diazadodecane (Thermospermine)	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$
1,13-Diamino-4,9-diazatridecane (Homospermine)	$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_4NH_2$
1,15-Diamino-4,8,12-triazapentadecane (Caldopentamine)	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$
1,16-Diamino-4,8,13-triazahexadecane (Thermopentamine)	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$
1,16-Diamino-4,8,12-triazahexadecane (Homocaldopentamine)	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$
1,19-Diamino-4,8,12,16-tetraazanonadecane (Caldohexamine)	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$
1,20-Diamino-4,8,12,16-tetraazaeicosane (Homocaldohexamine)	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$
Tris(3-aminopropyl)amine (Mitsubishine)	$[NH_2(CH_2)_3]_3N$
Tetrakis(3-aminopropyl)ammonium	$[NH_2(CH_2)_3]_4N^+$

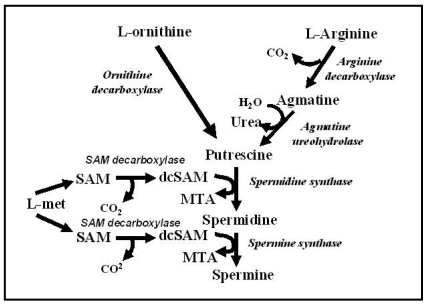

Fig. 1 Polyamine synthetic pathways found in bacteria and plants

Biosyntheses of Polyamines in *T. thermophilus*

In bacteria and plants, polyamines are synthesized from ornithine and/or arginine. Decarboxylation of ornithine produces putrescine and that of arginine produces agmatine. Agmatine can be converted to putrescine by the direct action of ureohydrolase or by the sequencial actions of deiminase and amidohydrolase as shown in Fig. 1. Spermidine and spermine are formed by aminopropylations from putrescine using

decarboxylated SAM (S-adenosylmethionine) as shown in the figure.

In contrast, *T. thermophilus* possesses a new polyamine metabolic pathway[4] as shown in Fig. 2. As shown in the figure, polyamines in the extreme thermophile are synthesized only from arginine. Agmatine formed from arginine is immediately aminopyropylated. In this pathway, spermidine is not synthesized from putrescine but from aminopropylagmatine. Recent studies disclosed that this new polyamine metabolism exists in other extreme thermophiles; two thermophilic arhaea[5,6].

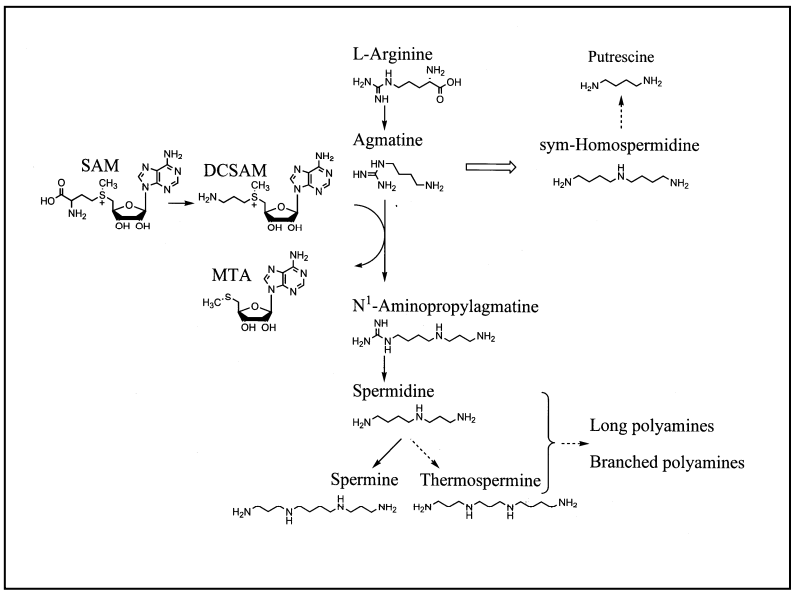

Fig. 2 Metabolic pathways in *T. thermophilus*. The dotted lines are suggested, but not yet confirmed. The reaction shown with ⇨ is being investigated.

Enigmas

We looked for *T. thermophilus* genes homologous to genes coding for aminopropyltransferases (also called spermidine synthase or spermine synthase). Only one gene (*speE* gene) was found as the homologous gene in *T. thermophilus* genome. The expression product of *speE* accepted only agmatine and triamines such as spermidine and caldine (nor-spermidine) as substrate, and the enzyme produces aminopropylagmatine, spermine and thermine. Thus the biosyntheses of other polyamines such as thermospermine, caldopetamine, homocaldopentamine, caldohexamine, homocaldohexamine, and tatrakis(3-aminopropyl)ammonium remain to be solved in our future studies.

Another enigma is biosynthesis of sym-homospermidine. The polyamine was detected as a minor component in the wild type cells. However, sym-homospermidine was the most abundant polyamine in cells of *speE* or *speD* (coding for SAM decarboxylase) knockout mutant. In these mutant cells, a large amount of sym-homospermidine was accumulated.

sym-Homospermidine synthesis

In bacteria and plants, sym-homospermidine is synthesized from either spermidine plus putrescine or two molecules of putrescine as shown in Fig 3. Both reactions are homologous to each other and is essentially homologous to deoxyhypusine synthase reaction in Eukaryotes[7]; a reaction in eIF5A synthesis. eIF5A seems to be an important protein for Eukaryotes and in yeast deletion of eIF5A is lethal. In plants, sym-homospermidine is an important starting material for their secondary metabolite syntheses.

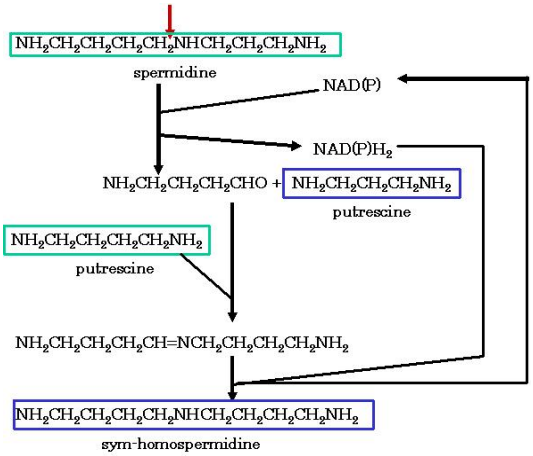

Fig. 3 Reaction mechanisms of sym-Homospermidine syntheses found in bacteria and plants.

We found a gene homologous to the gene (*dhs*) coding for deoxyhypusine synthase in *T. thermophilus* genome, and the knockout mutant was constructed. A double knockout mutant which lacks both *speE* and *dhs* could not produce sym-homospermidine indicating that the deoxyhypusine synthase homolog is involved in sym-homospermidine synthesis in *T. thermophilus*. However, Thermus *dhs* recombinant protein could not act as sym-homospermidine synthase *in vitro* suggesting that in *T. thermophilus*, sym-homospermidine synthesis reaction is unique and probably (a)other protein(s) is(are) required in addition to Dhs protein. The investigation is under way.

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