

Structural Specificity of the Triamines
sym-Homospermidine and Aminopropylcadaverine in Stimulating
Growth of Spermidine Auxotrophs of Escherichia coli

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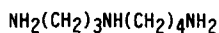
Received October 17, 1983

SUMMARY: sym-Homospermidine (HSpe) was compared with its structural isomer, aminopropylcadaverine (AP5), and the naturally-occurring triamine, spermidine (Spe), in its ability to stimulate the growth of Spe auxotrophs of Escherichia coli. HSpe was taken up by the cells, but was less effective than Spe in stimulating growth. In at least one E. coli auxotroph, HSpe was also less effective than AP5.

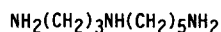
The triamine spermidine (Spe) is present in wild type Escherichia coli at millimolar intracellular concentrations. Using auxotrophic strains, it has been amply demonstrated that this amine is required for optimal growth (reviewed in reference 1), but not absolutely required for viability (2). In defining the molecular mechanisms of Spe action, it is of interest to understand the structural aspects of the molecule that are necessary for its growth-stimulatory role. We previously studied structural analogues of Spe in which the aminobutyl portion of the molecule (Figure 1) was lengthened. Increasing the chain length to 7 or 8 carbons resulted in no ability to stimulate growth and the C₅ and C₆ homologues showed intermediate behavior (3). Similar specificity was found with animal cells depleted of Spe by biosynthetic inhibitors (4). In the present study, we investigated the influence of lengthening the aminopropyl portion of the Spe molecule by one methylene group, as is found in sym-homospermidine (HSpe) (Figure 1). We found that HSpe was less effective than Spe and, in at least one E. coli auxotroph, also less effective than its structural isomer, aminopropylcadaverine (AP5).

METHODS AND MATERIALS

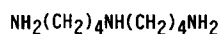
Bacterial strains. The wild type strain used in these studies is E. coli K12 strain 3000; this strain has only a thiamine requirement for growth. Strain



Spermidine (Spe)



Aminopropylcadaverine (AP5)

sym-Homospermidine (HSpe)Figure 1. *Structures of Triamines*

EWH319 is a K12 strain that was kindly provided by Dr. C. W. Tabor. EWH319 has 3 deletions influencing polyamine biosynthesis (2); one removing speA and speB, one spanning speC and glc and a third removing speD. Strain DR112 was constructed by cotransducing the spe(AB) deletion from EWH319 with serA into E. coli 3000 serA. DR112 showed considerably better growth when supplemented with spermidine than did EWH319 (see Table I). The poor growth of EWH319 appears to be due to the speC/glc deletion, since cotransductants containing both the spe(AB) and speC/glc deletions showed the slower growth characteristic of EWH319.

Cell culture. For culturing cells, the morpholinopropane sulfonic acid (MOPS) buffered medium of Neidhart et al. (5) was used together with an amino acid mix (6), 0.2% glucose, 13.2 mM K_2HPO_4 , and 10 μg thiamine/ml. Mutant strains were maintained on polyamine-supplemented media containing 5 μg Spe/ml. Prior to experimentation, polyamine starvation was accomplished by overnight growth in the absence of added polyamines through 10-13 generations. Following starvation, cultures were transferred to fresh medium containing Spe (as the trihydrochloride, CalBiochem), HSpe (a gift from Dr. Carl Porter), AP5 (synthesized as described in reference 3), or no polyamine. Final concentrations of the amines were as indicated below.

To insure against exogenous polyamine contamination, all glassware used for cell growth and harvest, or for analysis of polyamine content, was washed in a concentrated H_2SO_4 - $\text{Na}_2\text{Cr}_2\text{O}_7$ solution and rinsed in triple distilled water. In addition, all media and nutrient solutions were prepared with triple distilled water. To assess the level of trace polyamines, lyophilized samples of medium components were analyzed as described below. Neither Spe nor putrescine were present in quantities sufficient to stimulate growth (<5 picamoles per ml).

Cells in exponential growth from polyamine-starved cultures were used to inoculate medium in the presence or absence of polyamines at an initial density of $A_{450} = 0.005$ to 0.008. Routine supplementation of the triamines was as follows: 2 μg Spe/ml, 2 μg AP5/ml, and 0.125 or 1.25 μg HSpe/ml. These concentrations in the culture medium gave comparable intracellular levels, as shown in Results.

Cells were cultured at 37° C with vigorous rotary shaking and growth was monitored by measuring the turbidity at A_{450} . At $A_{450} = 0.095$ to 0.105, samples of 25 ml were taken into 30-ml tubes and centrifuged at 12,000 g for 3 min. Cells were washed twice with 10 ml of 0.9% NaCl to remove adsorbed amines. The cellular pellet was then vortexed with 0.5 ml of 0.2 N perchloric acid and stored overnight at 4° C.

Polyamine analysis. Cell extracts were centrifuged ca. 2 min at 12,000 g to remove debris and dansylated according to the method of Seiler (7) with modifications reported by Dreyfuss et al. (8). Chromatographic separations were

carried out on 0.2 mm plates of Silica Gel 60 (Merck). Solvents were selected which allowed best separation of Spe from its analogue:

- (i) Spe only: anhydrous diethyl ether/cyclohexane (3:1).
- (ii) Spe from AP5: benzene/methanol (9:1).
- (iii) Spe from HSpe: benzene/triethylamine (1:1).

Following separation, plates were examined under ultraviolet light and the appropriate spots were eluted with methanol. Quantitative assessment was made by conventional fluorometry (for a review see reference 9).

RESULTS

Polyamine content. The polyamine contents of two auxotrophic strains of *E. coli* are given in Table I. Initial experiments were performed (data not shown) to determine the appropriate initial concentrations of amines in the culture medium that would give rise to comparable intracellular levels. In

Table I
Triamine Contents and Growth Rates of Polyamine Auxotrophs

Polyamine Added	Concentration in Medium ^a	Intracellular Level ^b		Doubling Time ^c
		Spermidine	Analogue	
Strain DR112				
None	--	0.6	--	130
Spe	2.0	6.5	--	45
AP5	2.0	ND ^d	4.9	55
HSpe	0.125	0.4	3.9	130
HSpe	1.25	0.7	20.6	102
Strain EWH319				
None	--	ND	--	92
Spe	2.0	3.2	--	55
AP5	2.0	ND	3.5	62
HSpe	0.125	ND	2.7	80
HSpe	1.25	ND	3.9	61

^aμg/ml

^b(nmoles/ml)/A₄₅₀; corrected for adsorption to cell surface as described in the text.

^cmin

^dND (not detectable) = <0.06 nmoles per ml per A₄₅₀.

order to determine the degree of nonspecific external binding of each triamine, cultures grown in the absence of polyamines were cooled to 0° C and then supplemented with the appropriate amines at the concentrations indicated in Table I. Since polyamines are not transported into *E. coli* at 0° C (10), amounts remaining with cells after harvesting and washing (see Methods and Materials) were assumed to be adsorbed to the surface. The amount of triamine remaining adsorbed to the outer surface of the cell following harvesting and washing was 4-5% of the total cell-associated value for each homologue (data not shown). This is in close agreement with the values found previously (10) and has been subtracted to yield the values reported in Table I.

Strain EWH319, which contains deletions in all four genes coding for the enzymes of putrescine synthesis (see Methods and Materials), contained no detectable putrescine or Spe, when no polyamines were added to the culture media. Strain DR112, on the other hand, showed trace levels of Spe. This was presumably due to leakage through ornithine decarboxylase (11) which is present in this strain (see Methods and Materials).

Both auxotrophic strains took up the added triamines from the medium. Surprisingly, HSpe was transported more efficiently than Spe, indicating that it may be a better substrate for the Spe transport system. Higher levels of HSpe in the medium were not tested, so upper limits of HSpe accumulation are not known. In strain DR112, the presence of HSpe did not significantly influence the residual level of Spe.

Influence of Spe analogues on growth rate. The optical density of actively growing cultures was monitored to determine growth rates in the presence or absence of polyamines. Growth was generally monitored over at least a ten-fold increase in cell density. The growth responses are shown in Table I. When Spe was present in the medium, strain DR112 grew at a three-fold higher rate than if grown in the absence of Spe. As reported previously (3), AP5 was slightly less effective than Spe in stimulating growth. Raising the concentration of AP5 in the medium did not increase either the growth rate or the intracellular

concentration of the triamine (data not shown). On the other hand, the structural isomer of AP5, HSpe, was much less active than either Spe or AP5 in enhancing the rate of growth, even at three-fold higher intracellular levels. The culture grown with 1.25 μg HSpe/ml was slightly enhanced in growth over starved cells, but this may have been a result of slightly elevated Spe levels.

E. coli strain EWH319 demonstrated less ability to distinguish between the various polyamines. All three triamines were able to increase growth with nearly equal efficacy when present at similar intracellular levels. It should be noted, however, that strain EWH319 grew considerably more slowly than strain DR112. This was probably due to the large deletion spanning speC and glc in this strain, which is absent in DR112 (see Methods and Materials).

It is possible that HSpe was less effective than Spe (or than AP5 for strain DR112) because it inhibited growth in addition to substituting for Spe. In order to test this possibility, experiments with wild type E. coli 3000 were carried out. This strain synthesizes sufficient Spe to meet its metabolic requirements yet actively takes up triamines from the culture medium. In the absence of either analogue the level of intracellular Spe was 11 ± 0.5 nmoles per ml per A₄₅₀. When HSpe was added to the culture medium at an initial concentration of 1.25 $\mu\text{g}/\text{ml}$, intracellular levels of the analogue reached 4.8 nmoles per ml per A₄₅₀ without negatively affecting growth. Increasing the concentration of HSpe ten-fold did not effect either the growth rate or increase the intracellular level of the triamine. Wild type E. coli also took up AP5 from the medium, but less efficiently than HSpe. A level of AP5 of 1.1 nmoles per ml per A₄₅₀ was reached when it was supplied at 2 $\mu\text{g}/\text{ml}$; there was no effect on the rate of growth. HSpe, therefore, does not appear to inhibit the growth of wild type E. coli when present in cells at a level comparable to that of Spe.

DISCUSSION

The studies reported here with strain DR112 clearly demonstrate that HSpe is less effective than either its structural isomer AP5 or than Spe itself in enhancing growth. A similar result was obtained with the multiply-deleted mutant EWH319; however, results with this strain are somewhat compromised by the

existence of a defect, probably residing in the speC/glc deletion and apparently unrelated to polyamine biosynthesis, that limits the growth rate. The situation in E. coli is to be contrasted with that in animal cells where HSpe is fully as effective as Spe in supporting growth (4). Since HSpe occurs naturally in plants (12), it may be that the structural requirements at the aminopropyl end of the Spe molecule differ somewhat between eukaryotes and prokaryotes.

It is assumed that the specificity observed with Spe analogues arises in specificity of structural interactions rather than in metabolism. The only known metabolites of Spe in E. coli are its acetyl and glutathionyl conjugates; these are formed only under non-growing conditions (reviewed in reference 13). We found no evidence from the thin-layer chromatograms in this study for conjugates of Spe and its analogues under the culture conditions employed here. In the most widely considered model for Spe binding to DNA (14), N⁵ and N¹⁰ (the distance lengthened in AP5) bind across the narrow groove, while N¹ and N⁵, the spacial relationship which is altered in HSpe, bind to adjacent phosphates on the same strand. On the other hand, recent X-ray studies of DNA crystals suggest that in the crystalline state the polyamine spermine spans the major groove of the DNA double helix (15), rather than the minor groove as in the earlier model. Regardless of the specifics, these models for polyamine binding to DNA illustrate how the spacial relationship of the Spe amino groups could be important for its biological interactions.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the National Science Foundation (PCM-8301985).

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