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Structural Specificity of the Spermidine Requirement of an *Escherichia coli* Auxotroph

CAROLINE M. JORSTAD, JOHN J. HARADA, AND DAVID R. MORRIS*

Department of Biochemistry, University of Washington, Seattle, Washington 98195

A homologous series of spermidine analogs was synthesized with the general structure $NH_3^+(CH_2)_nNH_2^+(CH_2)_3NH_3^+$, where spermidine has $n = 4$. The influence of these compounds on growth and on the syntheses of protein and messenger ribonucleic acid was examined in a spermidine auxotroph of *Escherichia coli*. All of the homologs tested were taken up by the cells to an intracellular level equivalent to the level of spermidine which gives optimal growth. With increasing chain length of the homologs, there was reduced ability to stimulate growth. The homologs with $n = 7$ and $n = 8$ were essentially inactive. A similar specificity was observed when the ability of the homologs to restore the rates of protein and messenger ribonucleic acid chain elongation was compared to that of spermidine. These results suggest that a definite spatial arrangement of the amino groups of spermidine is required for productive interaction at its intracellular site(s) of action.

Wild-type *Escherichia coli* growing in minimal medium contains millimolar intracellular concentrations of spermidine [$NH_3^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$] and its precursor putrescine [$NH_3^+(CH_2)_4NH_3^+$] (30). Mutants of *E. coli* that are defective in most of the enzymatic steps of putrescine and spermidine biosynthesis have been isolated (2, 7, 17, 27). The physiology of these polyamine-deficient cells has been of interest in recent years (16). Starvation for polyamines reduced the growth rate of the mutant cells and produced an identical reduction in the rates of RNA and protein syntheses (14). Inhibition of RNA and protein syntheses was due to decreased polymerization rates rather than to specific effects only on initiation (9, 15). The rate of DNA replication fork movement was also slowed during polyamine deficiency (6). As an approach to further define the biological role(s) of spermidine, we examined the structural specificity of this requirement for optimal growth and macromolecular synthesis. In the work reported here, we synthesized a series of spermidine homologs of the general structure $NH_3^+(CH_2)_nNH_2^+(CH_2)_3NH_3^+$. All of the homologs tested were taken up by the mutant cells, and specificity was observed for support of growth and the rates of synthesis of RNA and protein.

MATERIALS AND METHODS

Spermidine homologs. The homologs of spermidine used in this study (Table 1) were synthesized by the procedure of Israel et al. (8). The appropriate diamine was reacted with redistilled acrylonitrile (Matheson, Coleman & Bell). The monocyanoethyl derivatives synthesized by this procedure were collected by distillation, and their purities were tested by thin-layer chromatography on cellulose plates (13). Reduction was carried out in a Parr hydrogenator by using Raney sponge nickel 28 (W. R. Grayson Co.) as a catalyst. After distillation, the product was dissolved in absolute ethanol, and the hydrochloride salt was collected after bubbling HCl gas through the solution. The identities of the products were confirmed by elemental analysis (Table 1). No ninhydrin-positive contaminants were detected by thin-layer chromatography (13) or by ion-exchange chromatography (19; see below). The diamine starting materials were purchased from the following sources: 1,5-diaminopentane and 1,8-diaminooctane, Matheson, Coleman & Bell; 1,6-diaminohexane, Ames Laboratories; and 1,7-diaminoheptane, Aldrich Chemical Co. The 1,5-diaminopentane and 1,6-diaminohexane were distilled before use; the others were used directly.

Bacterial strain and culture conditions. *E. coli* strain DK6 was constructed by transduction of a *speA* mutation (biosynthetic arginine decarboxylase) into *E. coli* strain AS19 (18). Strain AS19 was originally isolated for its sensitivity to actinomycin D, but was subsequently found to be sensitive to a spectrum of antibiotics, including fusidic acid, rifampin, and streptolydigen (23). Cultures were starved by overnight growth in the absence of polyamines, as previously described (18). The biochemical basis of this starvation has been discussed extensively (16, 17). The starved cultures were diluted and were grown with shaking at 37°C for at least three generations with or without polyamine supplementation in the culture medium described by Neidhardt et al. (21) supplemented with 0.2% glucose and a synthetic amino acid mixture (22). Routine polyamine supplementation was as follows: spermidine, 0.4 to 0.6 μg/ml; AP5, 0.3 to 0.6 μg/ml; AP6, AP7, and AP8, 25 to 100 μg/ml. These polyamine concentrations in the culture medium resulted in comparable intracellular levels (see below).

TABLE 1. *Spermidine homologs tested*

Designation	Structure[a]	Elemental analysis (%)							
		C		N		H		Cl	
		Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed
AP5	$NH_2(CH_2)_5NH(CH_2)_3NH_2$	35.96	35.66	15.73	15.55	8.99	8.68		
AP6	$NH_2(CH_2)_6NH(CH_2)_3NH_2$	38.43	38.44	14.95	14.78	9.25	9.42	37.37	37.31
AP7	$NH_2(CH_2)_7NH(CH_2)_3NH_2$	40.68	40.54	14.24	14.09	9.49	9.28	35.59	35.49
AP8	$NH_2(CH_2)_8NH(CH_2)_3NH_2$	42.72	42.61	13.59	13.50	9.71	9.83	33.98	33.91

[a] The amines were prepared as the hydrochloride salts.

Cellular composition. The cellular contents of protein, RNA, and DNA were analyzed as previously described (18). For measurement of polyamines, 30-ml cultures at a density of 10^8 cells per ml were poured onto ice with 10 mM (final concentration) NaN_3 to stop growth and to prevent acetylation of spermidine. The cells were collected by centrifugation. Polyamine pools were extracted as previously described (19). Our procedure for analysis has been described previously (19) and was modified by using a new resin system (29). All of the triamines were separated in this analytical system with appropriate alterations in the ionic strengths of the elution buffers (Table 2).

Polysome analysis. Polysomes were extracted and analyzed as previously described (9) with the following modifications. The culture medium was changed to that described above (21, 22). The efficiency and reproducibility of lysis were improved by the addition of sodium deoxycholate to 0.05% (wt/vol) after the freeze-thaw regimen.

β-Galactosidase induction. Growth of the cells, enzyme induction with isopropyl thiogalactoside, sampling into streptolydigen-containing medium, and the β-galactosidase assays were performed exactly as previously described (15). Enzyme units were defined as nanomoles of *o*-nitrophenyl galactoside hydrolyzed per minute and were normalized to 1 ml of culture at an absorbance at 450 nm of 1.0.

RESULTS

Polyamine content, growth, and macromolecular composition. Strain DK6 was grown in the presence of each of the spermidine homologs (Table 1). The levels of cell-associated putrescine, spermidine, and the particular homolog in question were measured (Table 3). Putrescine was not detected under any of the growth conditions, which is consistent with the mutational defect in arginine decarboxylase (18). Cells grown in the absence of a polyamine addition contained approximately 10% of the normal level of spermidine, as found previously (18). All of the spermidine homologs were taken up by the cells, although there were considerable differences in the efficiency of uptake. AP5 and spermidine reached equivalent intracellular levels at similar exogenous concentrations. However, the cells concentrated AP6, AP7, and AP8 much less efficiently; exogenous levels of at least 25 μg/ml were required to give intracellular polyamine concentrations similar to those obtained with spermidine at a concentration of 0.4 μg/ml. The polyamine determinations reported in Table 3 were performed on acid-hydrolyzed samples. Omitting the acid hydrolysis did not alter the results for spermidine, AP5, and AP8. The values for AP6 and AP7 in unhydrolyzed samples were lower by 40 and 15%, respectively, suggesting partial derivatization of these compounds by the cells.

Although the above results suggested that similar levels of cell-associated spermidine and its homologs could be reached by providing appropriate concentrations in the culture medium, the cellular location of the various triamines might differ. Spermidine uptake by *E. coli* con-

TABLE 2. *Buffers and elution times for amine analysis*

Second buffer[a]	Elution time (min)					
	Putrescine	Spermidine	AP5	AP6	AP7	AP8
1.5 N K^+	24	49	55			
2.4 N K^+	22	44		54	63	
4.0 N K^+	22	44				62

[a] All buffers were pH 5.6 and contained a molar ratio of KCl to potassium citrate of 26.7. Buffers are designated by their total normality of potassium ion. In all analysis runs, the first buffer contained 0.5 N K^+. At 30 min, a change was made to the indicated second buffer. The elution times are from the start of the first buffer.

TABLE 3. *Polyamine content and growth rate*

Polyamine added[a]	Polyamine concn (μg/ml of culture medium)	Polyamine content (nmol/mg of protein)[b]		Growth rate[c]
		Spermidine	Homolog	
None		5.2		0.95
Spermidine	0.4	47		2.00
AP5	0.6	3.1	52	1.62
AP6	100	2.3	57	1.36
AP7	50	2.1	44	1.04
AP8	25	1.7	37	0.91

[a] See text for details of growth conditions.
[b] The putrescine limit in all cultures was <0.2 nmol/mg.
[c] Expressed mass doublings per hour.

sists of two components, a rapid process not dependent on metabolic energy, which is complete in less than 2 min, and a slower, energy-dependent uptake (25). The first process was thought to be simple adsorption to the cell surface since it occurred at 0°C and the adsorbed material was rapidly exchangeable (25). To test the extent of adsorption in these experiments, starved cells of strain DK6 were treated with 10 mM NaN_3 at either 37 or 0°C and then exposed to spermidine or its homologs for 5 min at the concentrations indicated in Table 3. In all cases, the levels of cell-associated triamines were less than 10% of those reported in Table 3. Thus, the levels attained in growing cells were not due simply to adsorption to the cell surface. In addition, an assessment of the distribution of spermidine and its homolog AP6 in cell extracts was made by differential centrifugation. Strain DK6 was grown in the presence of either 0.3 μg of spermidine per ml or 25 μg of AP6 per ml. The two cultures were treated with NaN_3 and mixed, and the cells were harvested. Lysates were prepared by the lysozyme–freeze-thaw method (see above for polysome analysis) without the use of detergents, treated with DNase, and fractionated into a low-speed (30,000 × *g*, 30 min) pellet and a high-speed (70,000 × *g*, 17 h) upper supernatant, lower supernatant, and pellet. The distribution of cellular spermidine in these fractions was 7, 22, 38, and 33%, respectively. The distribution of AP6 was very similar (7, 22, 44, and 27%, respectively). Although these results do not necessarily reflect intracellular localization (see below), it seems clear that the spermidine homolog AP6 was internalized by the cells and was distributed similarly to spermidine in the fractions separated by differential centrifugation.

The growth rates of strain DK6 in the presence of similar cellular levels of the various triamines are compared in Table 3. In the absence of added amines, the growth rate was twofold lower than that observed in the presence of spermidine, as reported previously (18). Cells grown with putrescine in the culture medium grew at a rate identical to that observed with spermidine (specific growth rate, 2.0 doublings per h) (data not shown). The longer-chained triamines (AP7 and AP8) did not significantly enhance growth, although the intracellular concentrations were similar to the spermidine levels giving optimal growth. AP7 and AP8 were tested in the range from 10 to 200 μg/ml and had no effect on the growth of the starved cells, except for a significant inhibition at the higher concentrations of AP8. AP5 and AP6, also at intracellular levels equivalent to spermidine levels in spermidine-supplemented cultures, gave intermediate enhancement of growth, with AP5 being more effective than AP6 (Table 3). Raising the concentration of AP5 in the medium (10 μg/ml) increased the growth rate of the cells significantly (specific growth rate, 1.8 doublings per h). Thus, AP5 at sufficiently high intracellular concentrations seems to be nearly as effective as spermidine in fulfilling the polyamine requirement of this auxotroph. AP6 was not tested at concentrations higher than the 100 μg/ml reported in Table 3.

It is clear that none of the homologs can fully replace the spermidine requirement at equivalent intracellular concentrations. However, quantitative interpretation of these differences in growth rates is difficult since supplementation with the homologs reduced the basal cellular spermidine content as much as threefold below the level in starved cells (Table 3).

In these experiments, exponential growth was routinely observed over an 80- to 160-fold increase in cell mass after addition of the homolog. In other experiments, where subculturing was continued over a period of several days, no change in growth rate was observed until revertants prototrophic for putrescine synthesis appeared in the cultures. Hence, it appears that the cells maintain steady-state growth indefinitely in the presence of the homologs, albeit at lower rates.

The macromolecular composition of strain DK6 grown in the presence of the various triamines is shown in Table 4. For the measurements described here, cells were starved for polyamines as described above. The cultures were then grown with the indicated polyamine supplementation for at least an 80-fold increase in mass before sampling. Duplicate measurements made approximately one mass doubling apart agreed within experimental error, indicating that a steady-state situation existed during the course of the experiment. The RNA content of strain DK6 was unaltered by polyamine starvation or by growth in the presence of the homologs. The invariance in the RNA/protein ratio during pol-

TABLE 4. *Macromolecular composition*

Polyamine added[a]	RNA concn (μg/μg of protein)	DNA concn (μg/10^9 cells)	Protein concn (μg/10^9 cells)
None	0.47	19	400
Putrescine	0.49	21	350
Spermidine	0.48	25	630
AP5	0.50	19	480
AP6	0.48	15	315
AP7	0.50	18	430
AP8	0.50	18	490

[a] See text for details of growth conditions.

yamine limitation of growth has been described previously (18). This is a reflection of the fact that cellular ribosome content is constant during polyamine starvation, but the rate of protein synthesis per active ribosome decreases (9). In addition, the DNA contents of the cells showed only minor differences under the various growth conditions. However, examination of cell size (i.e., protein content) revealed an interesting property. Although there were only small variations in the protein contents of starved cells, putrescine-grown cells, and cells grown in the presence of the homologs, the spermidine-grown cells were larger by about 50%. Strain DK5, which is the parent of DK6 and is prototrophic for polyamine synthesis (18), had a protein content of about 400 $\mu g/10^9$ cells under these growth conditions. Thus, stimulation of growth by spermidine in the absence of intracellular putrescine led to an increase in cell size.

Ribosome distribution. Control cultures grown in the presence of putrescine or spermidine were labeled with [^{14}C]uracil. Starved cultures or cultures grown with the spermidine homologs were labeled with [^{3}H]uracil and then mixed with ^{14}C-labeled control cells. Lysates were analyzed by sucrose gradient centrifugation. To illustrate the type of profiles observed, an experiment with AP8 is shown in Fig. 1. The polysome patterns from control and AP8-grown cells were similar (Fig. 1A). All of the radioactive material in the polysome region of the gradient was converted to 70S monosomes by treatment with pancreatic RNase (data not shown). The region consisting of subunits and monosomes is displayed in more detail in Fig. 1B. Other than a small increase in the ratio of subunits to monosomes in the AP8-grown cells, there were no meaningful differences in this region of the gradient between the two cultures. The data from experiments in which each of the homologs was tested are summarized in Table 5. As the length of the homolog increased, there was a slight decrease in the amount of ribosomal material in polysomes and a corresponding increase in the subunit region. Using these data on the fraction of ribosomes in polysomes, together with the cellular growth rates (Table 3), the cellular RNA/protein ratio (Table 4), and the value of 87% of total RNA as rRNA (18), we calculated polypeptide chain elongation rates for cells under the various culture conditions (Table 5). As was found previously (9, 15), starvation for polyamines reduced the rate of polypeptide chain elongation in a manner similar to the decrease in growth rate. The spermidine homologs showed a specificity similar to that observed for restoration of growth (Table 3). Thus, AP5 and

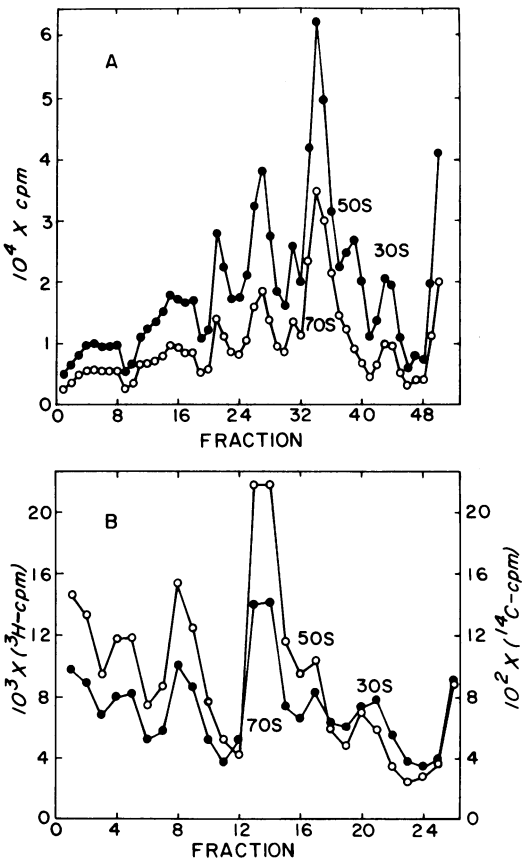

FIG. 1. *Sucrose density gradients comparing patterns of polysomes and ribosomal subunits from cells grown in the presence of spermidine and AP8. The cells were grown as described in the text. [^{14}C]uracil-labeled cells* (○) *grown in the presence of spermidine were mixed with [^{3}H]uracil-labeled cells* (●) *grown with AP8 supplementation. (A) For analysis of polysomes, centrifugation was for 145 min at 36,000 rpm in a Beckman SW41 rotor, using a 10-ml gradient of 15 to 30% sucrose over a 1-ml cushion of 45% sucrose; 6.15 × 10^5 cpm of ^{14}C and 1.18 × 10^6 cpm of ^{3}H were applied to the gradient. (B) For analysis of monosomes and subunits, centrifugation was for 185 min at 45,000 rpm in a Beckman SW50.1 rotor, using a 4.6-ml gradient of 15 to 35% sucrose over a 0.2-ml cushion of 80% sucrose; 7.16 × 10^4 cpm of ^{14}C and 5.64 × 10^5 cpm of ^{3}H were applied to the gradient.*

AP6 stimulated the elongation rate, but were less effective than spermidine. AP7 and AP8 were essentially inactive.

Induction of enzyme-forming capacity. We previously showed that polyamine starvation slowed the rate of β-galactosidase messenger chain elongation (15). To estimate the influence of the spermidine homologs on this parameter, we measured the time from the addition of

TABLE 5. *Ribosome distributions and calculated polypeptide elongation rates*

Polyamine added[a]	Ribosome distribution[b]			Elongation rate[c]
	Polysomes	Monosomes	Subunits	
None	69	12	19	8.2
Spermidine	71	12	17	14.9
AP5	66	12	22	13.7
AP6	66	14	20	12.0
AP7	61	12	27	8.8
AP8	59	11	30	8.4

[a] See text for details of growth conditions.

[b] Expressed as percentage of total ribosomal material.

[c] Amino acids per second per growing chain. Calculated as described by Forchhammer and Lindahl (4) by using the formula $[(P/120) \times \mu \times \ln 2]/[(R/1.6 \times 10^6) \times f]$, where P and R are protein and rRNA contents of the cells in micrograms per milligram (dry weight), respectively, μ is the specific growth rate in mass doublings per hour, and f is the fraction of ribosomes active in protein synthesis. The value for f was calculated by assuming that all of the polysomes and one-half of the monosomes were active in protein synthesis.

inducer to the first appearance of active message for β-galactosidase. After induction, cultures were sampled at timed intervals into solutions containing the antibiotic streptolydigen to block further RNA synthesis (1, 15, 23). The samples were further incubated to allow expression of completed message, and β-galactosidase was then assayed (Fig. 2). The induction lags in starved and unstarved cells were similar to those observed previously (15). The four homologs were less effective than spermidine in shortening the lag period; their effectiveness decreased with increasing chain length.

To interpret these results, it is necessary to know whether polyamine starvation or supplementation of the cells with the homologs changed the rate of action of the inducer. In wild-type *E. coli*, induction of the lactose operon began simultaneously with the addition of isopropyl thiogalactoside (10) and was not influenced by polyamine starvation (15). This was tested in cells grown in the presence of AP8 (Fig. 3). In this experiment, transcriptional initiation was blocked with rifampin at various times before and after addition of isopropyl thiogalactoside at zero time. The cultures were then incubated for completion and expression of previously initiated mRNA and then assayed for β-galactosidase. In the AP8-grown culture, as was found previously for polyamine-starved cells (15), the accumulation of rifampin-resistant, enzyme-forming capacity extrapolated back to zero time. Identical results were obtained with AP5, AP6, and AP7. Thus, growth in the presence of the homologs introduced no lag in inducer action.

Since the inducer acts essentially instantaneously under the conditions tested in this research, it is possible to estimate the rates of message elongation from the induction lags (15). The average values for the induction lags from three independent experiments are listed in Table 6. A length for the *lacZ* gene of 3,063 nucleotides was estimated from the length of the polypeptide chain (5), and rates of mRNA elongation were calculated. The values of 28 and 42 nucleotides per s in starved and unstarved cells, respectively, were in agreement with previously reported values (15). As was observed qualitatively in Fig. 2, the values for the spermidine homologs were intermediate between the two extremes.

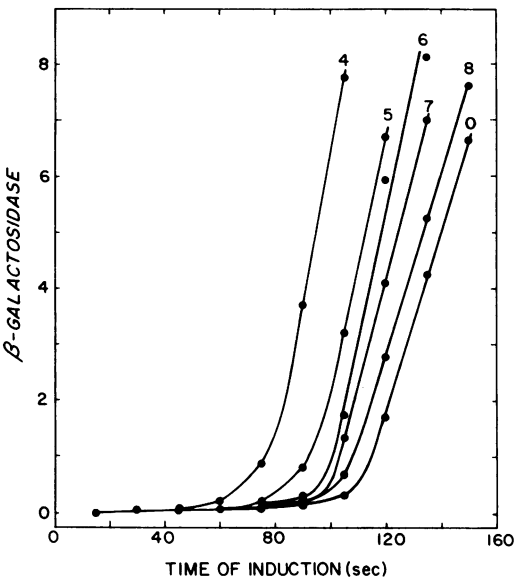

FIG. 2. *Kinetics of production of streptolydigen-resistant, enzyme-forming capacity. Cultures were grown and induced with isopropyl thiogalactoside at zero time (see text). At the indicated times, samples were taken into streptolydigen, incubated for 20 min at 35°C for expression of enzyme-forming capacity, and assayed (15). The basal, uninduced levels (0.88 to 1.46) were subtracted to allow better comparison of the curves. Curve 0 is for the starved culture, curve 4 is for the spermidine-supplemented culture, and curves 5 through 8 are for cultures grown with homologs AP5 through AP8.*

DISCUSSION

Strain DK6 requires spermidine or its biosynthetic precursor putrescine for optimal growth. The object of the present study was to examine the structural constraints of this requirement by modifying the spermidine molecule. In order for these results to be meaningful, the physiological effects of the spermidine homologs would have to be compared with the effects of spermidine at

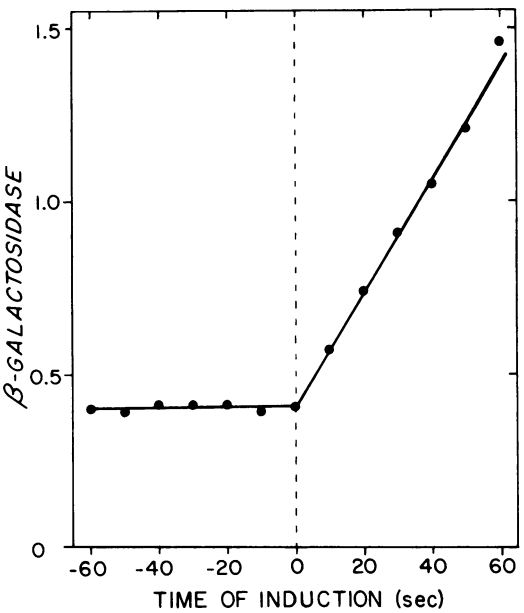

FIG. 3. *Formation of rifampin-insensitive enzyme-forming capacity in cells grown in the presence of AP8. Rifampin (60 μg/ml) was added to samples of the culture at the times indicated on the abscissa, and isopropyl thiogalactoside was added at zero time. The samples were then incubated at 35°C for 15 min for expression of enzyme-forming capacity and assayed for β-galactosidase.*

TABLE 6. *β-Galactosidase induction lags and calculated mRNA elongation rates*

Polyamine added[a]	Lag (s)[b]	Elongation rate (nucleotides/s)[c]
None	110	28
Spermidine	73	42
AP5	85	36
AP6	95	32
AP7	98	31
AP8	104	29

[a] See text for details of growth conditions.

[b] The values were derived from back extrapolation of the final slopes of induction curves such as those shown in Fig. 2. Values are the averages of three independent experiments.

[c] Calculated as described in the text.

equivalent intracellular concentrations. This was achieved by appropriately adjusting the concentrations of the polyamines added to the culture medium. Our results revealed a definite specificity for growth. Lengthening the C_4 methylene chain of spermidine decreased the growth-promoting activity of the triamine, with the C_7 and C_8 compounds being essentially devoid of biological activity. When the influence of the triamines on protein and RNA syntheses was examined, a qualitatively similar activity series was observed. This specificity could have arisen in one of two ways. First, there may be a requirement for a certain spatial arrangement of the amino groups of spermidine for productive interaction at its cellular site of action. Alternatively, the least active homologs might be unavailable for interaction at the appropriate cellular sites, either because of covalent conjugation or because of an altered intracellular localization. Neither of the latter two possibilities seems likely. Analysis of hydrolyzed and unhydrolzyed samples revealed no correlation between apparent degree of conjugation and ability to restore growth. Examination of the distribution of spermidine and AP6 by differential centrifugation of cell lysates failed to detect a significant difference. It should be emphasized that redistribution of the triamines would be expected to an undefined extent in the combined lysate (25). However, the important point is that the two compounds redistributed identically; AP6 showed no evidence of sequestration by or selective affinity for subcellular components. In addition, the homologs showed no specificity for adsorption to the cell surface. Thus, the most likely explanation of our results at present is that the specificity for the triamines arose at the site(s) of spermidine action.

It is of interest that a qualitatively similar activity series was observed for the influence of the triamines on growth and on the syntheses of RNA and protein. These results could be consistent with the triamines exerting their effect on these three parameters at a single intracellular site of action. As discussed previously (15), this site could be either protein or RNA synthesis, because of the coupling between the two processes. Thus, stimulation of either transcription or translation could affect the other processes. Alternatively, spermidine may exert its growth-promoting activity through interaction at multiple sites in the cell. In this case, the similar specificity for stimulation of protein and RNA syntheses might suggest a unifying fundamental interaction of spermidine in the two processes, for example binding of the triamine to nucleic acids (see below).

The natural occurrence of *sym*-homospermidine (11) and of *sym*-norspermidine (AP3) (3, 24, 31) leads one to suspect that there is a certain latitude in the spatial arrangements of the amino groups of spermidine as far as its biological function is concerned. The fact that AP3 was equivalent to spermidine in restoring the growth of polyamine-starved *E. coli* cells was consistent with this possibility (18). This paper describes the first systematic study of this question. It

seems clear that extending the C_4 methylene chain to C_5 and longer reduces the biological activity of the molecule. This may be related to the mode of binding of the spermidine molecule to DNA. Liquori and co-workers (12) proposed a model for spermidine binding to DNA in which N^5 and N^{10} interact with phosphate residues on opposite sides of the narrow groove and N^1 and N^5 bind to phosphates on the same strand. Lengthening the C_4 methylene chain would alter the interactions across the groove and thus might give rise to the altered biological properties of the homologs.

Although this paper has addressed specifically the function of spermidine, the intracellular concentration of putrescine, its biosynthetic precursor, can be higher than the concentration of the triamine by 3- to 10-fold in wild-type *E. coli* (30). Despite this high level of putrescine, there are several lines of evidence suggesting that spermidine is the physiologically more important of the two in terms of cell growth. Strain DK6 grows at the wild-type rate with undetectable intracellular levels of putrescine (Table 3). This same conclusion was reached previously with another mutant strain of *E. coli* (17). Although the growth of *E. coli* in the absence of putrescine seems normal, there appear to be some physiological abnormalities. The protein content of wild-type *E. coli* and of mutant cells grown in the presence of putrescine is considerably less than that when growth of the mutant is stimulated by spermidine. This suggests a possible role for putrescine or for the ratio of putrescine to spermidine in determining cell size at division when the cultures are growing at wild-type rates. On the other hand, at suboptimal growth rates (either starved cells or growth with the homologs) cell size is normal in the absence of putrescine. Munro and Bell (20) showed that with auxotrophs in culture media of low osmolarity, putrescine allowed wild-type growth, whereas cultures containing spermidine grew more slowly. It is not known whether this possible role of putrescine in osmotic balance is related to the effect on cell size. In any event, these results are highly suggestive of a physiological role for putrescine different from that of spermidine.

Can putrescine alone fulfill the polyamine requirement in *E. coli*? The answer is probably no, at least not with equal efficiency. Mutants with a complete block in *S*-adenosylmethionine decarboxylase have no detectable intracellular spermidine and a 70% increase in putrescine over the wild-type level (27). These cells show a reduction in growth rate of approximately 25%, which is restored to normal by the addition of spermidine. Thus, it appears at present that putrescine and spermidine may play their own specific roles in *E. coli*, with some overlapping specificity. However, this situation may differ considerably with different microbial species, since there is a wide range of phylogenetic variation in amine contents (28).

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