Effects of Various Triamines on Cell-Free Polypeptide Synthesis of *Escherichia coli* and on Growth of Its Polyamine Auxotrophs

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The effects of various synthetic triamines having a general structure, $H_2N(CH_2)_xNH(CH_2)_yNH_2$, where $x=2$—5 and $y=2$—8 (abbreviated, x–y; with 3–4 being spermidine itself), on poly(U)-directed polypeptide synthesis of *Escherichia coli* and on growth of its polyamine-requiring mutants were examined in comparison with those of spermidine. Except for 2–2 and 2–3, all of the triamines stimulated more or less polypeptide synthesis at suboptimal Mg^{2+} concentrations, but the Mg^{2+} concentration required for the maximal stimulatory effect was different for each triamine. The degree of maximal stimulation caused by 3–3 (norspermidine), 4–4 (homospermidine), or 4–5 was nearly comparable with that by spermidine. The acetylspermidines were inactive, however, they inhibited the spermidine-stimulated polyphenylalanine synthesis. Many of the triamines examined reduced the ratio of leucine to phenylalanine incorporation into polypeptides during poly(U)-directed translation, and the degree of this effect did not necessarily correspond with that of the stimulatory effect. Moreover, 2–4, 2–5, 3–3 and 4–4 could stimulate the growth of a polyamine auxotroph of *E. coli*, MA 261, as effectively as did spermidine. However, 3–3 was the only triamine which could fully replaced spermidine in promoting growth of a mutant strain, KK 101, which is more dependent on polyamines than MA 261. Thus, these results demonstrated that some synthetic triamines were as active as spermidine in eliciting these effects, and also that there were some differences among these effects in the structural requirement for triamine.

Keywords spermidine analog; cell-free polypeptide synthesis; stimulatory effect; translational fidelity; growth-promoting effect; *Escherichia coli*; polyamine auxotroph

The polyamine, spermidine, is an aliphatic polyvalent cation ubiquitously present in all living cells.[1] There is now considerable evidence that it is involved in different steps of protein synthesis, both in prokaryotic and eukaryotic cell-free systems.[1b,2] The *in vitro* experiments have demonstrated that spermidine has a stimulatory effect, which can not be fulfilled by any amount of Mg^{2+} in its absence, on overall protein or homopeptide synthesis *in vitro* at suboptimal Mg^{2+} concentrations.[3] The effect can entail an increase in translational fidelity.[4] The step at which spermidine mainly affects the rate and fidelity of polyphenylalanine synthesis has been defined to be in the binding of aminoacyl-transfer ribonucleic acid (tRNA) to ribosomes.[4d] Moreover, it has been shown from the experiments using polyamine-deficient bacteria that these cells contain abnormal small ribosomal subunits with undermethylated 16S RNA and a decreased content of S1 ribosomal protein,[5] which seem to initiate protein synthesis with a reduced efficiency.[6]

In view of its high positive charge at physiological pH, spermidine very likely exerts many of its effects through direct interaction with anionic sites of the machinery for protein synthesis. Recently, Kakegawa *et al.* have shown that spermidine avidly binds to ribosomal RNA (rRNA).[7] In defining the spermidine action to both the *in vivo* and *in vitro* biological events, it is of considerable importance and interest to learn the structural aspects of the spermidine molecule that are necessary for its various roles. The structural modifications such as alteration in the length of polymethylene chains separating the nitrogen atoms would be one of the approaches to better understand the interaction between spermidine and its anionic binding sites. In line with this, several experiments using the synthetic spermidine analogs having an aminopropyl moiety[$H_2N(CH_2)_3NH(CH_2)_nNH_2$, $n=3$—8] have been conducted to evaluate their effects on *in vitro* protein synthesis[8] and on growth of polyamine-deficient mutants of *Escherichia coli*.[9]

In the present work, we examined a series of synthetic triamines [$H_2N(CH_2)_xNH(CH_2)_yNH_2$; $x=2$—5 and $y=2$—8] for their effects on *in vitro* polypeptide synthesis of *E. coli* and on growth of two kinds of its polyamine auxotrophs, and compared their effects with those caused by spermidine.

Materials and Methods

Materials L-[U-^{14}C]Phenylalanine [405 mCi (15 GBq)/mmol] and L-[U-^{14}C]leucine [270 mCi (10 GBq)/mmol] were purchased from ICN; adenosine triphosphate (ATP) (Tris salt), guanosine triphosphate (GTP) (Tris salt), polyuridylic acid [poly(U)] (K salt), phosphocreatine (Tris salt), creatine phosphokinase and *E. coli* tRNA [Type XXI, acceptor activity (pmol/A_{260} unit): 133 for phenylalanine and 72 for leucine] from Sigma; and spermidine trihydrochloride and bentonite from Wako Pure Chemicals. The latter was purified according to the procedure of Fraenkel-Conrat *et al.*[10] Triamines used in this study have the general structure of $H_2N(CH_2)_xNH(CH_2)_yNH_2$ and are abbreviated as x–y. Diethylenetriamine (2–2), *N*-(2-aminoethyl)-1,3-diaminopropane (2–3), 3,3′-iminobispropylamine (3–3) and 3,3′-diamino-*N*-methyl-dipropylamine (3–3-Me) as the free amines were obtained from Aldrich, and were converted to the corresponding hydrochlorides, which were recrystallized from methanol/water. Stock solutions of the amines were adjusted to pH 7.0. Other chemicals used were of the highest purity commercially available. The polyamine auxotrophs of *E. coli*, MA 261 (*speB speC serA thr leu thi*)[11] and KK 101, which was constructed from MA 261 by mutagenesis,[12] were kindly provided by Dr. K. Igarashi.

Syntheses of Triamines and Monoacetylated Derivatives Triamines ($x=2$—5, $y=4$—8) were synthesized by the alkylation of diamines with ω-bromoalkylphthalimide followed by the hydrolysis with 12 M HCl according to the method described by Okada *et al.*,[13] but *N*-(5-bromopentyl)-phthalimide was synthesized by replacing dimethylformamide with acetone as the solvent. N^1- and N^8-acetyl derivatives of spermidine were synthesized by the methods of Tabor *et al.*[14] The comparable methods were used for the syntheses of N^1-acetyl derivatives of 3–3 and 4–4 from monoacetylated 1,3-diaminopropane and putrescine, respectively. All amines synthesized were purified by chromatography on a column of Dowex 50W × 8 with a linear gradient of HCl (0—4.5 M)[13] followed by recrystallization from ethanol or 1-propanol/*n*-hexane. The identity and purity of each amine synthesized was confirmed by gas chromatography-mass spectrometry (GC-MS)[15] and thin-layer[13] and paper chromatography.[16] No contaminating materials which provided

GC peaks and reacted with ninhydrin were present in any preparation.

Preparation of S-30 The 30000 × *g* supernatant (S-30) was prepared from exponentially growing cells (A_{520} 0.15—0.18) of *E. coli* K-12 as described by Nirenberg,[17] except that cells were disrupted in a French press in the presence of purified bentonite (50 mg/ml). The S-30 fraction was not preincubated, but was dialyzed at 4 °C for 12 h against three changes of 100 vols. buffer (pH 7.6) containing 100 mM Tris–HCl, 15 mM magnesium acetate, 100 mM ammonium chloride, 6 mM mercaptoethanol in order to exclude endogenous polyamines. After dialysis, insoluble materials were removed by centrifugation to obtain a clear S-30 fraction, which was stored in small portions at −80 °C. The concentrations of putrescine and spermidine in the S-30 fractions determined by GC method[18] were approximately 1.4 and 8.5 μM, respectively. Protein concentration of the S-30 was determined by the Lowry method with bovine serum albumin as the standard.

Assay Methods for Poly(U)-Directed Polyphenylalanine Synthesis and Leucine Misincorporation The reaction mixture (0.5 ml), which was constructed on the basis of the previous reports,[4b,19] contained 50 mM Tris–HCl (pH 7.6), 100 mM ammonium chloride, 6 mM mercaptoethanol, 2.5 mM ATP, 0.25 mM GTP, 5 mM phosphocreatine, 40 μg creatine phosphokinase, 40 μg *E. coli* tRNA, 100 μg poly(U), 100 μg S-30 protein (25 μl), 1 μM L-[^{14}C]phenylalanine (0.2 μCi), magnesium acetate and triamine at the specified concentration. After incubation at 37 or 47 °C for 15 min, the reaction mixture was treated according to Lubin and Ennis.[19a] The hot trichloroacetic acid insoluble radioactivity on a Millipore HA filter was assayed with a liquid scintillation spectrometer. Under these conditions, polyphenylalanine synthesis was linear up to 20 min. The misincorporation of leucine into polypeptide was measured under the following conditions: the same reaction mixture for poly(U)-directed polyphenylalanine synthesis was used, but L-[^{14}C]phenylalanine was replaced by 1 μM L-[^{14}C]leucine (0.135 μCi) and 1 μM cold phenylalanine. All the reported experiments were repeated more than three times with similar results. The counts of each sample were corrected for those of a blank without poly(U).

Growth of *E. coli* Mutant Strains MA 261 and KK 101 The growth medium and the procedure for obtaining polyamine depleted bacteria were the same as those of Kashiwagi *et al.*[12] The starved cells inoculated at an initial cell density of 0.03 (A_{540}) were grown in a minimal medium supplemented with either spermidine or triamine as indicated. The growth-promoting effect was assayed by comparing the growth, measured at 540 nm, 24 h after inoculation.

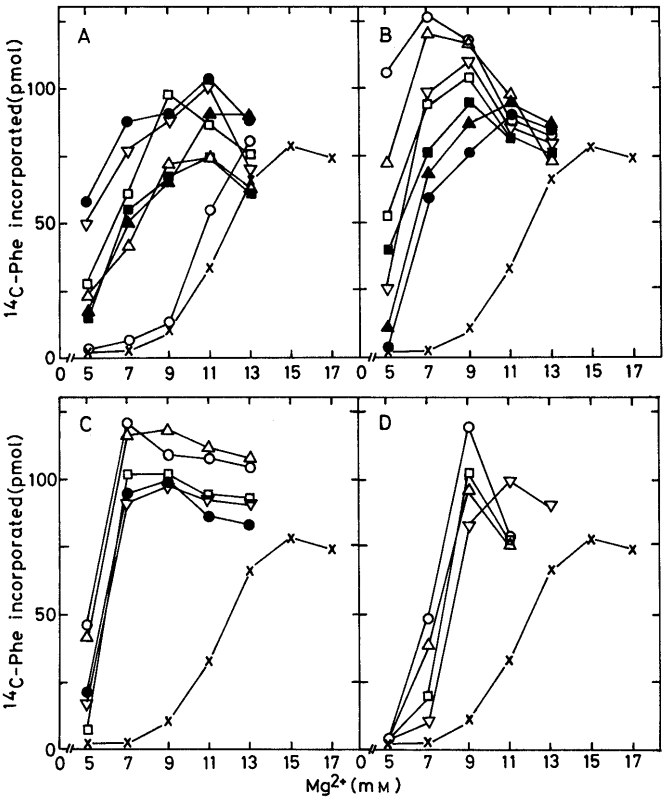

Fig. 1. Effect of Triamines on Polyphenylalanine Synthesis in an *E. coli* Cell-Free System at 37 °C

Incubation was carried out under standard conditions in the presence of 2 mM triamine. The triamines, $H_2N(CH_2)_xNH(CH_2)_yNH_2$, are abbreviated as *x–y*. (A): ○, 2–2; ■, 2–3; □, 2–4; ▽, 2–5; ●, 2–6; ▲, 2–7; △, 2–8. (B): ○, 3–3; △, 3–4 (spermidine); □, 3–5; ▽, 3–6; ●, 3–7; ▲, 3–8; ■, 3-3-Me[$H_2N(CH_2)_3N(CH_3)(CH_2)_3NH_2$]. (C): ○, 4–4; △, 4–5; □, 4–6; ▽, 4–7; ●, 4–8. (D): ○, 5–5; △, 5–6; □, 5–7; ▽, 5–8; ×, without added triamine.

Results

Effect of Triamines on the Rate of Polyphenylalanine Synthesis at Various Mg^{2+} Concentrations First, we carried out the experiments to determine the optimal concentrations of spermidine and Mg^{2+} needed for stimulating polyphenylalanine synthesis at 37 or 47 °C in our system. The incubation temperatures of 37 and 47 °C were adopted, since the stimulation of polypeptide synthesis by polyamines has been reported to be further enhanced by elevating the incubation temperature.[8b] The results obtained showed that the stimulatory effect at 37 °C was largest at 2 mM spermidine and 7 mM Mg^{2+}, amounting to about 1.6-fold stimulation over an optimal Mg^{2+} concentration (15 mM) without spermidine. Similarly, the optimal concentrations of spermidine and Mg^{2+} for stimulation at 47 °C were derermined to be 3 and 9 mM, respectively. Under these conditions the stimulatory effect increased by about 2-fold, being in line with the observation of Kakegawa *et al.*[8b] Therefore, the concentration of the triamines tested in the following experiments was fixed to 2 mM at 37 °C or 3 mM at 47 °C, and their stimulatory effects were examined by varying the Mg^{2+} concentration.

The results upon incubation at 37 °C are shown in Fig. 1. Among the triamines tested, only 2–2 had no effect on polyphenylalanine synthesis and 2–3 and 2–8 exhibited only a weak sparing effect on Mg^{2+} requirement (Fig. 1A). However, 2–4, 2–5 and 2–6 produced stimulation more than 80% of that caused by spermidine, although the Mg^{2+} concentration required for the maximum effect was relatively high. The naturally occurring triamine, 3–3, was nearly equal to spermidine both in the degree of stimulation and in the requirement of Mg^{2+} (Fig. 1B). As seen for 3–3-Me, the substitution of the central imino hydrogen of 3–3 with a methyl group lowered the stimulatory effect with a shift of the optimal Mg^{2+} concentration to 9 mM. This is possibly attributable to steric constraints which hinder its binding to the anionic sites. Similarly, 3–5 and 3–6 had a maximum stimulation at 9 mM Mg^{2+}, this higher Mg^{2+} requirement than with spermidine being compatible with the results of Igarashi *et al.*[8b] In general, the Mg^{2+} requirement of the triamines containing an aminopropyl group for their maximum stimulation increased as the number of the methylene group in another side chain increased. Among the triamines with an aminobutyl group, the higher homologs (4–4, 4–5, 4–6, 4–7 and 4–8) were unique in that they were active at a relatively wide range of Mg^{2+} concentrations (Fig. 1C). At 7 mM Mg^{2+}, 4–4 (naturally occurring triamine) and 4–5 were nearly as effective as spermidine. Clearly differing from other triamines having an aminopentyl group, 5–5, 5–6 and 5–7 revealed a sharp maximum of the stimulation at 9 mM Mg^{2+} (Fig. 1D). The following triamines significantly,

but not maximally, stimulated polypeptide synthesis at 7 mM Mg^{2+}, where spermidine showed a maximum effect: 2–6, 3–3, 3–5, 3–6, 4–4, 4–5, 4–6, 4–7 and 4–8.

Incubation at 47 °C brought about a more significant increase in stimulation than that at 37 °C, but this was accompanied by the increase in the optimal Mg^{2+} concentration. Figure 2 shows the results of the triamines which exhibited relatively high stimulation at the same Mg^{2+} concentration as spermidine (9 mM). Interestingly, the degree of stimulation by 2–4 and 2–5 increased remarkably compared with that at 37 °C, and was nearly comparable with that by spermidine.

Effect of Monoacetyl Derivatives of Spermidine, 3–3 and 4–4 on Polyphenylalanine Synthesis Monoacetylation of polyamines has been well documented in both prokaryotes[1b] and eukaryotes.[20] The functional role of this reaction has been assumed to be as a regulatory mechanism for a variety of polyamine actions and appropriate maintenance of their cellular concentrations of free polyamines.[20] Mezl *et al.*[21] have shown that the inhibitory effect of sermidine on a eukaryotic translation system at an optimal Mg^{2+} concentration is mostly abolished by N^1-acetylation of this amine. However, according to our knowledge, there is no report as to how the acetylspermidines themselves affect on polypeptide synthesis. Then, N^1- and N^8-acetylspermidines as well as N^1-acetylated derivatives of 3–3 and 4–4 were examined for their effects on polypeptide synthesis. It was found that they were virtually inactive in the range of 2–20 mM at suboptimal Mg^{2+} concentrations, at least underlining that the terminal nitrogen atoms able to have positive charges are indispensable for stimulation by the triamines. Nevertheless, addition of either of the acetylspermidines to the incubation mixture inhibited the stimulation induced by spermidine. As shown in Fig. 3, N^1-acetylspermidine was somewhat

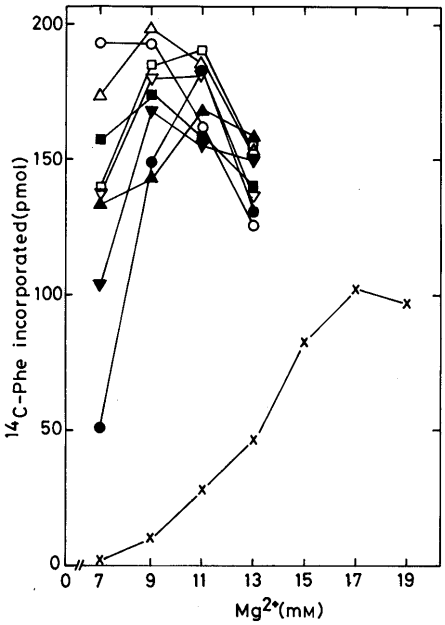

Fig. 2. Effect of Triamines on Polyphenylalanine Synthesis in an *E. coli* Cell-Free System at 47 °C

Incubation was carried out under standard conditions in the presence of 3 mM triamine. Abbreviations: see Fig. 1. ▽, 2–4; □, 2–5; ●, 2–6; ○, 3–3; △, 3–4 (spermidine); ▲, 3–5; ■, 4–4; ▼, 4–5; ×, without added triamine.

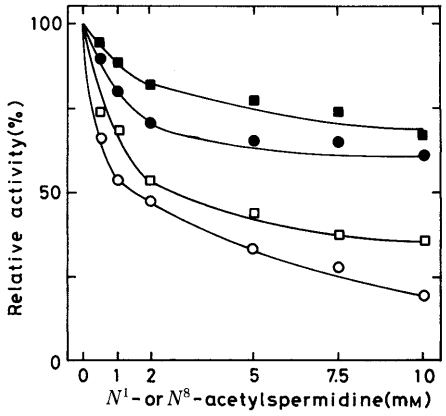

Fig. 3. Inhibitory Effect of Acetylspermidines on Spermidine-Stimulated Polyphenylalanine Synthesis in an *E. coli* Cell-Free System

Incubation was carried out at 37 °C under standard conditions in the presence of 1 mM (open symbols) or 2 mM (closed symbols) spermidine. The amounts of phenylalanine incorporated in the absence of acetylspermidines (111.2 pmol at 2 mM spermidine and 54.1 pmol at 1 mM spermidine) were expressed as 100%. ○ and ●, N^1-acetylspermidine; □ and ■, N^8-acetylspermidine.

TABLE I. Effect of Triamines on Leucine Misincorporation during Poly(U)-Directed Polyphenylalanine Synthesis in an *E. coli* Cell-Free System[a]

Triamine added[b] (2 mM)	Mg^{2+} concentration[c] (mM)	^{14}C-Phe incorporated (pmol)	Stimulation by triamine (-fold)	^{14}C-Leu incorporated (pmol)	$\frac{\text{Leu}}{\text{Phe}} \times 100$
—	15	78.3	—	7.4	9.5
2–4	9	98.1	1.25	2.8	2.9
2–5	11	100.0	1.28	9.3	9.3
2–6	11	103.8	1.33	8.1	7.8
3–3	7	126.3	1.61	7.8	6.2
3–4 (spermidine)	7	120.3	1.54	2.8	2.3
3–5	7	101.2	1.29	1.4	1.4
3–6	9	109.5	1.40	4.0	3.7
4–4	7	120.8	1.54	1.9	1.6
4–5	9	118.6	1.51	2.8	2.4
4–6	9	102.3	1.31	1.2	1.2
4–7	9	97.8	1.25	3.1	3.2
4–8	9	99.4	1.27	7.3	7.3
5–5	9	119.6	1.53	2.9	2.4
5–6	9	96.1	1.23	3.4	3.5

a) Incubation was carried out at 37 °C under standard conditions. Radioactivity without poly(U) was subtracted from each value. *b*) Abbreviations: see Fig. 1. *c*) At the Mg^{2+} concentration indicated, each triamine showed the maximum stimulatory effect.

more effective than N^8-acetylspermidine in inhibiting the spermidine effect. The K_i values for N^1- and N^8-acetylspermidines calculated by the Dixon plots were 2.9 and 3.3 mM, repsectively.

Effect of Triamines on Leucine Misincorporation during Poly(U)-Directed Translation Under our experimental conditions the ratio of leucine to phenylalanine incorporation into polypeptides in the presence of Mg^{2+} alone (15 mM) was higher than that reported by Igarashi *et al.*[4b] However, the ratio of phenylalanine- to leucine-tRNA formation, which was determined by incubating the standard reaction mixture, without poly(U), at 37 °C for 15 min and then by measuring the radioactivity of cold 5% trichloroacetic acid insoluble material, was almost identical to that of the acceptor activity (phenylalanine/leucine = 1.85) of *E. coli* tRNA used, at least suggesting that the aminoacylation level was not responsible for this high misincorporation of leucine. The addition of spermidine at 2 mM significantly decreased the percentage of leucine incorporation compared to phenylalanine (Table I). The similar degree of decrease in leucine misincorporation has been reported for an *E. coli* system with spermidine.[22] Then, the triamines, which had significantly stimulated polypeptide synthesis, were examined for their effect on leucine misincorporation. As shown in Table I, many of the triamines tested brought about a remarkable decrease in leucine misincorporation. Moreover, examination of these triamines at the Mg^{2+} concentration 2—3 mM higher than those optimal for the stimulatory effect showed a tendency to increase the leucine misincorporation. This was in agreement with the observation for spermidine.[4b] It was interesting to point out that, despite its highly stimulatory effect, 3–3 provided only a little decrease in leucine misincorporation.

Effect of Triamines on Growth of Polyamine-Dependent Mutants The effect of the triamines, which had showed the significant stimulation on polypeptide synthesis, on the growth of two kinds of polyamine auxotrophs of *E. coli* was investigated (Table II). Each of the triamines was added to the growth medium at a final concentration of 100 μM, at which spermidine exhibited the maximum growth-promoting effect, although there might be considerable differences in the efficiency of uptake among the triamines tested. The strain KK 101 has been shown to be more strongly dependent on polyamines than MA 261.[12] In MA 261, 2–4, 2–5, 3–3 and 4–4 could fully replace spermidine, and 3–5 was active but to a lesser extent. However, in the case of KK 101, only 3–3 was as effective as spermidine, and 2–4 and 4–4 showed the activity 59% and 83%, respectively, of that observed for spermidine.

TABLE II. Effect of Triamines on Growth of Polyamine Auxotrophs of *E. coli*

Amine added[a]	Growth of auxotroph[b]	
	MA 261	KK 101
3–4 (spermidine)	1.00	1.00
None	0.43	0.18
Putrescine	1.15	1.15
2–4	1.05	0.59
2–5	1.07	0.17
2–6	0.37	0.13
3–3	1.02	1.07
3–5	0.88	0.21
3–6	0.56	0.14
4–4	0.96	0.83
4–5	0.44	0.17
4–6	0.09	0.05
4–7	0.13	0.08
4–8	0.08	0.13
5–5	0.13	0.03
5–6	0.10	0.03

a) Putrescine and the triamine were added at 1 mM and 100 μM, respectively. Abbreviations: see Fig. 1. *b*) The growth (absorbance at 540 nm) was compared after 24 h culture. The absorbance was 0.83 for MA 261 and 0.75 for KK 101 in the presence of spermidine, which were expressed as 1.00. Values are the averages of triplicate experiments.

Discussion

From the comparative studies of various synthetic triamines with spermidine, it appeared that most of them stimulated, to various degrees, polypeptide synthesis of *E. coli* at suboptimal Mg^{2+} concentrations. This may suggest that both nonspecific and specific interactions between the triamines and ribosomal anionic sites are operative. However, among the triamines tested, only 3–3, 4–4 and 4–5 were equivalent to spermidine both in stimulating polypeptide synthesis and in sparing the Mg^{2+} requirement, suggesting that these triamines might bind to ribosomes with the same specificity as spermidine. The stimulatory effect by triamine was decreased by increasing the Mg^{2+} concentration, as typically seen in spermidine. Mg^{2+} probably competes with the triamines in their binding sites, although the exact mechanism of this phenomenon is not clear. However, 4–4 and 4–5 did not show such a tendency, suggesting that a quite different mechanism of action may be operative in the interaction between these triamines and Mg^{2+}. Alternatively, these triamines could bind through the aminobutyl moiety to ribosomes so strongly that they might not be replaced by Mg^{2+}. It seems reasonable that rRNAs are also the sites of action of the other triamines tested in this study, but the results showed that the spatial relationship of the triamine amino groups is very critical for their interactions of biological importance. The significant stimulation by the triamines having a longer methylene chain than C_3 or C_4 in the spermidine molecule can be accounted for by bending back these triamine molecules which then makes their binding to ribosomes possible or by reaching to alternative binding sites. However, this probably causes the distortion in the ribosomal conformation detrimental to the expression of the stimulatory effect. On the other hand, it should be noted that 2–2 was ineffective. The close distance of the imino and amino nitrogen in 2–2 would produce the charge repulsion and consequently prevent the imino nitrogen from having a charge,[23] so that it might behave more like diamine than triamine under the incubation conditions used (pH 7.6). This is in accordance with the finding that the N^1-acetylation of 3–3, spermidine and 4–4 resulted in a complete disappearance of their stimulatory effects at suboptimal Mg^{2+} concentrations.

It has been shown that N^1-acetylspermidine is more active than N^8-acetylspermidine in provoking the B–Z transition of polynucleotides[24] and in increasing the melting temperature of deoxyribonucleic acid (DNA).[25] This indicates that these acetylated compounds still have the nucleotide binding activity. Thus, it seems likely that

inhibition of spermidine stimulation by its acetylated derivatives is mainly due to their partial competition for spermidine binding. However, it should be stated with caution whether the physiological function of the acetylspermidines resides in regulating the spermidine action, since the relatively high concentration of these compounds was required for their effects.

As in a previous report,[8] stimulation by the triamines tested in this study was also temperature dependent. Raising the incubation temperature up to 47 °C may afford a certain latitude to the spatial arrangement of the anionic sites to which the triamines bind. This latitude may be preferentially favorable for binding of 2–4, 2–5 and 2–6, since these triamines were more effective than the others.

Many of the synthetic triamines examined were as effective as spermidine in decreasing the percentage of leucine incorporated compared to phenylalanine, suggesting that the structural specificity for triamine in the effect may not be pronounced. At present we cannot explain this phenomenon. In addition, the reason why 3–3 was less effective than spermidine in preventing the leucine misincorporation remains obscure. Further experiments are required to clarify the details on these subjects.

Very interestingly, it was found that in addition to 3–3 and 3–5 which have already been reported to be active,[9a,b] 2–4 and 2–5 also promoted the growth of MA 261 as effectively as did spermidine. Furthermore, 4–4 was nearly equal to spermidine in stimulating the growth of MA 261. This, however, was not consistent with the result of Morris *et al.*[9c] who reported that 4–4 is much less active than spermidine. The discrepancy is probably due to differences in the mutant strains used and/or in the amount added to the medium. These results indicated that the growth-promoting effect on MA 261 was restricted to the triamines containing 6—8 carbon atoms in total. However, in the case of KK 101, only 3–3 was as effective as spermidine in fulfilling its polyamine requirement. It has been reported[12] that, in addition to the depletion of putrescine biosynthetic enzymes, this strain has a certain dysfunction in the 30S subunits and thereby the association of S1 protein, which is preferentially produced soon after the addition of putrescine, to S1-depleted 30S subunits become weaker than this association in 30S subunits from MA 261. Therefore, the assembly of functionally active 30S subunits in KK 101 can be supported exclusively by spermidine and 3–3, indicating that, in this case, the structural requirement for triamine is very stringent.

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