Stabilization of nucleic acids by unusual polyamines produced by an extreme thermophile, *Thermus thermophilus*

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Extreme thermophiles produce two types of unusual polyamine: long linear polyamines such as caldopentamine and caldohexamine, and branched polyamines such as quaternary ammonium compounds [e.g. tetrakis(3-aminopropyl)ammonium]. To clarify the physiological roles of long linear and branched polyamines in thermophiles, we synthesized them chemically and tested their effects on the stability of ds (double-stranded) and ss (single-stranded) DNAs and tRNA in response to thermal denaturation, as measured by differential scanning calorimetry. Linear polyamines stabilized dsDNA in proportion to the number of amino nitrogen atoms within their molecular structure. We used the empirical results to derive formulae that estimate the melting

temperature of dsDNA in the presence of polyamines of a particular molecular composition. ssDNA and tRNA were stabilized more effectively by tetrakis(3-aminopropyl)ammonium than any of the other polyamines tested. We propose that long linear polyamines are effective to stabilize DNA, and tetrakis(3-aminopropyl)ammonium plays important roles in stabilizing RNAs in thermophile cells.

Key words: differential scanning calorimetry (DSC), nucleic acid, polyamine, thermal denaturation, tetrakis(3-aminopropyl)ammonium, *Thermus thermophilus*.

INTRODUCTION

Putrescine, spermidine and spermine are naturally occurring linear polyamines that are present at high concentrations in all growing cells [1,2]. The polyamines have been reported to be involved in a wide variety of cellular regulatory pathways, including those that control the rate and fidelity of macromolecule syntheses [3], the equilibrium between cellular proliferation and apoptosis [4] and the induction of cellular differentiation [2]. Since the cellular concentrations of these amines are much greater in actively dividing cells when compared with quiescent or resting cells, it is thought that polyamines are required for optimal growth of almost all cell types and that the increased biosynthesis of polyamines is necessary for cells to traverse the cell cycle [5]. In contrast, depletion of polyamine content through inhibition of their biosynthesis or mutation of key enzymes involved in their production results in significant inhibition of cell growth. At the same time, it has been reported that excessively high concentrations of polyamines can be toxic to cells [6].

Many organisms, including mesophilic bacteria, tend to produce only two or three of the polyamines, putrescine, spermidine and spermine, as major components. However, analysis of the polyamine profiles of thermophilic bacteria, including both eubacteria and archaeobacteria, has revealed a variety of new compounds [7,8]. In particular, an extreme thermophile (eubacterium), *Thermus thermophilus*, produces a variety of unusual polyamines [9,10]. In these cells, 16 unique and standard polyamines have been identified (Figure 1).

These include branched polyamines such as tetrakis(3-aminopropyl)ammonium, and long linear polyamines such as caldohexamine, which are often found in thermophiles as major polyamine components. The cellular concentrations of these longer and branched polyamines directly correlate with increases in the growth temperature of *T. thermophilus* [9], suggesting that

they play an important role in the maintenance of cellular function at high temperature. These polyamines are essential for protein synthesis *in vitro* at the physiological temperatures of the thermophiles' natural environment [11,12].

Polyamines bound to nucleic acids can induce aggregation or conformational changes of DNA and can stabilize DNA [13–17]. The molecular basis of the binding of polyamines to DNA has been investigated by a variety of techniques, such as NMR imaging [18–20], CD [21], Raman spectroscopy [22] and X-ray crystallography [23,24]. However, the precise nature of the interaction of polyamines with DNA has not yet been characterized in detail.

Our preliminary studies have demonstrated that the unusual polyamines in *T. thermophilus* stabilize the conformation of dsDNA (double-stranded DNA) at high temperatures [25]. To clarify the role of these unique polyamines in thermophiles, in the present study, we examined the effects of these compounds on the stability of nucleic acids using DSC (differential scanning calorimetry). We chemically synthesized unusual polyamines produced by *T. thermophilus*, since they are not available commercially. We used chemically synthesized DNA oligomers to measure the precise melting temperature of dsDNA. We also analysed the thermal denaturation of a single species of tRNA and an ssDNA (single-stranded DNA) with a stem-and-loop structure in the absence and presence of polyamines.

MATERIALS AND METHODS

Materials

Norspermidine · 3HCl, spermidine · 3HCl and spermine · 4HCl were purchased from Wako Chemical (Osaka, Japan). Thermine was purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.).

Abbreviations used: DSC, differential scanning calorimetry; dsDNA, double-stranded DNA; SSC, standard saline citrate; ssDNA, single-stranded DNA; tRNA^{Phe}, phenylalanine-specific tRNA.

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Figure 1 The 16 polyamines present in the cells of *T. thermophilus*

The number of carbon atoms separating the amino or aza groups is shown in parentheses. The nine underlined polyamines were used in this study.

Thermospermine, caldopentamine, homocaldopentamine, caldohexamine, tris(3-aminopropyl)amine (mitsubishine) and tetrakis(3-aminopropyl)ammonium were chemically synthesized according to methods described previously [26–28]. All the polyamines used were in the hydrochloride form. Complementary deoxyoligonucleotides of the sequence, 5'-GCGTCATACAGTGC-3' (S1) and 5'-GCACTGTATGACGC-3' (S2), were purchased from Amersham Biosciences (Little Chalfont, Bucks, U.K.). The sequences were chosen based on the rationale described in [29]. The 5'- and 3'-terminal sequences of S1 are complementary to each other and S1 forms a stem-and-loop structure in solution as shown in Figure 2. Likewise S2 forms a stem-and-loop structure, but the stem part is much shorter compared with the structure of S1. We confirmed that the melting temperatures of S1 and S2 are identical with those reported in [29]. tRNA^{Phe} (phenylalanine-specific tRNA) from brewer's yeast was purchased from Sigma–Aldrich. All other reagents used were purchased from Wako Chemical.

Thermal denaturation of DNA

All polyamines were dried overnight in a vacuum desiccator, weighed and dissolved in 0.1 × SSC (standard saline citrate; 15 mM NaCl and 1.5 mM sodium citrate), pH 7.0, at various concentrations. All solutions were filtered through 0.20 µm type

Figure 2 Secondary structure of a 14-mer ssDNA (S1)

Figure 3 Excess heat capacity curves observed for thermal denaturation of a 14 bp dsDNA in the absence (broken line) and presence (solid line) of various amounts of tetrakis(3-aminopropyl)ammonium

Curves are shifted on the y-axis for illustrative purposes. From left to right: all melting temperature experiments were conducted with tetrakis(3-aminopropyl)ammonium at concentrations ranging from 0.05 to 0.3 mM, in steps of 0.05 mM.

CE Minisart filters before use. Double-helical oligomers were obtained by mixing the two single-stranded oligonucleotides (S1 + S2) in 0.1 × SSC and heating to 100°C for 3 min. The solution was then cooled gradually to an appropriate temperature and used for the thermal denaturation studies. We confirmed that the thermal denaturation temperature (T_m) of the dsDNA is 52.6°C and T_m of the S1 ssDNA is 27.3°C in 0.1 × SSC, as reported previously [29]. Figure 3 shows that the dsDNA preparation used in the present study contained no detectable amount of ssDNA. On heating, only thermal denaturation of dsDNA was recorded, but no peak corresponding to thermal melting (27°C for S1 and 22°C for S2) was observed. Samples were prepared by mixing an equal volume of stock solutions of polyamines and DNA at known concentrations. The concentration of DNA was spectrophotometrically determined by evaluating the absorption of the solution at 260 nm on a Beckman DU-640 spectrometer (Beckman Instruments, Palo Alto, CA, U.S.A.). The concentrations of S1, S2 and dsDNA were estimated using the absorption coefficients of these nucleic acids reported previously [29]. The DNA (either S1/S2 dsDNA or S1 ssDNA) concentration was kept constant at approx. 110 µM for all experiments, whereas the amount of polyamine added to the mixtures was varied.

DSC

DSC was performed on a MicroCal (Microcal LLC, Amherst, MA, U.S.A.) MC-2 microcalorimeter equipped with a Haake F3 temperature-controlled water bath. All DNA samples (both dsDNA and ssDNA) were dissolved in 0.1 × SSC buffer. The scan rate used was 1 °C/min. Data were collected between 10 and

100 °C. Sample and reference cells were placed under 0.15 MPa dry N₂ during the scan. Each sample was scanned at least three times. The reversibility of thermal denaturation was confirmed in experiments with and without the addition of spermine. There was no difference between the thermograms of the first and the second runs.

Data were fitted using Origin (version 2.9) for DSC (MicroCal). Buffer-versus-buffer scan (baseline) was subtracted from each sample scan before plotting the thermogram. To estimate the enthalpy change, a baseline was fitted and the area above the baseline was integrated. T_m was taken to be the point at which change in apparent heat capacity is maximal. ΔH_{cal} is the enthalpy change of melting obtained directly from the total area under the composite DSC peak as applied in a previous study [29].

Thermal denaturation of RNA

All polyamines and MgCl₂ · 6H₂O were weighed and dissolved in 50 mM NaCl/10 mM sodium cacodylate buffer (pH 7.0) to a concentration of 0.2 mM. Stock solutions were prepared in autoclaved, diethyl pyrocarbonate-treated buffer. All buffers were filtered through 0.20 µm type CE Minisart filters before use. Brewer's yeast tRNA^{Phe} was dissolved in the same buffer. Samples were prepared by mixing an equal volume of stock solutions of a particular polyamine with tRNA^{Phe} of known concentrations. The concentration of tRNA^{Phe} was spectrophotometrically determined by evaluating the absorption of the solution at 260 nm on a Beckman DU-640 spectrometer assuming that the absorption coefficient is $5.3 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$ [30]. The concentration of tRNA^{Phe} was kept constant at approx. 30 µM for all experiments, whereas we varied the amount of polyamine added to the mixture.

The contaminated polyamines in tRNA^{Phe} used in this study were analysed. When 0.3 mM tRNA solution was applied to the polyamine analyser, less than 400 pmol/ml spermidine and less than 500 pmol/ml spermine were detected. These amounts are too small and negligible compared with the polyamines added to the tRNA in the present study.

The method of the DSC analysis was the same as that used for the experiments with DNA, although a different reference buffer was used. All samples were dialysed against 50 mM NaCl/10 mM sodium cacodylate buffer. The analysis of DSC data was performed as described in [31]. CURFIT analysis of the data was performed according to a non-two-state model for each component transition. Apparent T_m was taken to be the point at which change in apparent heat capacity is maximal.

RESULTS

Effects of unusual polyamines on the thermal stability of dsDNA

We investigated the effects of the unusual polyamines found in *T. thermophilus* on the stability of nucleic acids using DSC. We chose to evaluate the effects of these polyamines on double-helical (B-form) DNA (dsDNA), comprising the annealed S1 and S2 oligonucleotides. The G + C content of S1 and S2 is 57 %.

Nine polyamines, a triamine, a tetra-amine, a penta-amine, their homologues, a hexa-amine and two branched polyamines were chosen for DSC analyses among the 16 polyamines produced by *T. thermophilus*. These polyamines were chosen so as to compare systematically the effects of polyamines with different numbers of amino nitrogen atoms on the stability of nucleic acids. The chemical structures of these polyamines are shown in Figure 1.

Figure 3 shows the DSC profiles of dsDNA in the absence and presence of tetrakis(3-aminopropyl)ammonium at the indicated concentrations. The position of the endothermic transition peak

Figure 4 Effects of various polyamine concentrations on the melting temperature (T_m) of a 14 bp dsDNA

(A) Effects of polyamine concentrations on the melting temperature (T_m) of a 14 bp dsDNA. Variations in T_m values for norspermidine (○), spermidine (△), thermine (◇), spermine (●), caldopentamine (□), caldohexamine (▲), mitsubishine (◆) and tetrakis(3-aminopropyl)ammonium (■) are shown. (B) Relationship between T_m and number of nitrogen atoms of polyamines. Line A, polyamines containing a butyl group; line B, polyamines containing only propyl groups as carbon backbone. T_m values in the presence of branched polyamines are in parentheses. The upside-down open triangle is homocaldopentamine. The relationship between T_m and number of nitrogen atoms is determined at a polyamine concentration of 0.2 mM.

was shifted to a higher temperature with increasing concentrations of this polyamine, demonstrating that stabilization of the dsDNA was positively correlated with the concentration of tetrakis(3-aminopropyl)ammonium. A similar correlation was found for all polyamines tested as shown in Figure 4(A). The value of T_m increased in proportion to the concentration of polyamine, with the magnitude of the effect varying with the particular polyamine tested. After an initial sharp increase at a low concentration of polyamine, T_m profiles gradually stabilized, indicating a relatively strong affinity of polyamines for dsDNA.

Table 1 summarizes the thermodynamic parameters characterizing the thermal denaturation of dsDNA in the absence and presence of various polyamines at a concentration of 0.2 mM. Homocaldopentamine exhibited the greatest effect on dsDNA stability among the polyamines tested, leading to an increase in T_m of approx. 9.7 °C at 0.2 mM.

To compare the effect of the interaction of polyamines with dsDNA in greater detail, we compared the melting temperatures (T_m) of the DNA/polyamine mixtures at a fixed concentration of each polyamine. The T_m of the pure synthetic dsDNA increased linearly in proportion to the number of amino nitrogen atoms present within the chemical structure of the individual polyamines tested (Figure 4B). From Figure 4(B, line B), the following empirical formula was obtained for the T_m of the dsDNA in the presence of linear polyamines containing only aminopropyl groups at a concentration of 0.2 mM

$$T_m = 1.1N + 55.5$$

Table 1 Summary of the changes in melting temperature (T_m) and enthalpy (ΔH_{cal}) of 14 bp dsDNA in the presence of various polyamines at a concentration of 0.2 mM

T_m values were averaged for at least three experiments and the average errors in these values were $\pm 0.1^\circ\text{C}$.

Polyamines	No. of carbon atoms separating the amino or aza group	T_m ($^\circ\text{C}$)	ΔH_{cal} (kJ/mol)
None		52.6	421.1 ± 3.8
Norspermidine	33	58.6	518.5 ± 4.5
Spermidine	34	59.1	515.2 ± 4.7
Thermine	333	60.1	548.6 ± 3.9
Spermine	343	61.3	572.4 ± 7.2
Caldopentamine	3333	61.3	570.7 ± 4.9
Homocaldopentamine	3334	62.3	618.3 ± 7.5
Caldohexamine	33333	61.9	589.1 ± 9.1
Mitsubishine	3(3)3	59.0	527.4 ± 4.2
Tetrakis(3-aminopropyl)ammonium	3(3)(3)3	59.0	530.2 ± 6.2

where N is the number of amino and aza nitrogen atoms present in the polyamine.

On testing the same polyamines at a concentration of 0.3 mM, the following formula was obtained (graph not shown):

$$T_m = 1.6N + 54.9$$

It is interesting to note that the replacement of an aminopropyl group with an aminobutyl group in the chemical structure of the polyamine tested was associated with a proportional increase in the T_m of the dsDNA by approx. 0.9°C per aminobutyl group (Figure 4B, line A). These results suggest that both the total charge and the distance separating the positive charges are important to stabilize the dsDNA. Basu and Marton [13] have reported similar results when comparing DNA–polyamine interactions. The following formula was subsequently deduced from Figure 4(B, line A), to calculate the T_m of the dsDNA in the presence of 0.2 mM linear polyamine

$$T_m = 1.1N + 0.9B + 55.5$$

where N is the number of amino or aza nitrogen atoms and B is the number of butyl groups in the polyamine tested ($B = 0$ or 1). When 0.3 mM linear polyamine was used, the following formula was obtained:

$$T_m = 1.6N + 1.2B + 54.9$$

For the branched polyamines, mitsubishine and tetrakis(3-aminopropyl)ammonium (Figure 4B), the melting temperatures are lower than those obtained in the presence of linear polyamines containing the same number of nitrogen atoms, indicating that the branched polyamines are less effective in stabilizing dsDNA. For example, caldopentamine stabilized DNA much more effectively than tetrakis(3-aminopropyl)ammonium, despite the fact that both polyamines contained four propyl groups and five primary, secondary or quaternary amino nitrogen atoms, and despite the identity of their chemical formulae in a neutral solution ($\text{C}_{12}\text{H}_{36}\text{N}_5^+$). Similarly, thermine was more effective than mitsubishine, as shown in Table 1 and Figure 4(B). It is clear that long linear polyamines stabilize dsDNA more effectively than branched polyamines.

Enthalpy changes estimated from the thermogram were subsequently compared with the melting temperatures (Figure 5).

Figure 5 Temperature dependence of enthalpy change associated with the thermal melting of dsDNA in the presence of various polyamines

○, Norspermidine; △, spermidine; ◇, thermine; ●, spermine; □, caldopentamine; ▲, caldohexamine; ◆, mitsubishine; and ■, tetrakis(3-aminopropyl)ammonium.

Table 2 Summary of the melting temperature (T_m) of S1 ssDNA in the presence of various polyamines at a concentration of 0.3 mM

T_m values were averaged for at least three experiments and the average errors in these values were $\pm 0.1^\circ\text{C}$

Polyamines	No. of carbon atoms separating the amino or azo group	T_m ($^\circ\text{C}$)
None		27.3
Spermidine	34	29.3
Thermine	333	30.6
Spermine	343	31.3
Caldopentamine	3333	30.8
Tetrakis(3-aminopropyl)ammonium	3(3)(3)3	32.1

The change in enthalpy associated with the thermal denaturation of dsDNA increased proportionally with the melting temperature.

The effects of unusual polyamines on the thermal stability of ssDNA

We also evaluated the effects of the various polyamines on ssDNA using DSC and S1 in $0.1 \times \text{SSC}$. The first and last four nucleotides of S1 are capable of hybridizing with each other to form a stem-and-loop structure as shown in Figure 2. On heating in aqueous solutions, the stem part melts in a two-state fashion at a certain temperature T_m . S1 can be regarded as a simplified model of RNAs, which consists of many stem-and-loop structures. The position of the endothermic peak (T_m) was shifted to a higher temperature by the addition of the various polyamines as shown in Table 2. As with the thermal denaturation of dsDNA, the increase in T_m correlated with the length of the linear polyamine chains at a fixed concentration of 0.3 mM. In contrast with the results found for dsDNA, ssDNA was stabilized more effectively by tetrakis(3-aminopropyl)ammonium when compared with caldopentamine, which is a linear polyamine with the same chemical formula, suggesting that the branched polyamine is particularly effective at stabilizing stem-and-loop structures.

The effects of unusual polyamines on the thermal stability of tRNA

Figure 6 shows the DSC profiles of yeast tRNA^{Phe} in the absence and presence of various polyamines at a concentration of 0.2 mM. The endothermic transition peaks were recorded as described in the Materials and methods section. On binding of polyamines to tRNA^{Phe}, we observed an increase in the apparent T_m , as expected. The increase in the apparent T_m mediated by linear polyamines

Figure 6 Excess heat capacity curves of 0.2 mM tRNA^{Phe}

Experiments were conducted in the absence (A) and in the presence of Mg²⁺ (B), thermine (C), spermine (D), caldopentamine (E), caldoxamine (F), mitsubishine (G), tetrakis(3-aminopropyl)ammonium (H).

was accompanied by an increase in the change in enthalpy at a fixed concentration of 0.2 mM as shown in Table 3.

T_m increased proportionally with an increase in the chain length of the linear polyamines as in the case of dsDNA. However, two of the branched polyamines stabilized tRNA^{Phe} more effectively than the corresponding linear polyamines with the same molecular formula. For example, tetrakis(3-aminopropyl)ammonium stabilized tRNA^{Phe} more efficiently than caldopentamine and all other polyamines tested.

The deconvolution of thermograms was performed using CURFIT algorithm as reported previously [31]. Table 3 summarizes the enthalpy changes on thermal denaturation of tRNA^{Phe}. The results of the decomposition for a non-two-state model of the melting curves are shown in Figure 7, and the sum of the change in enthalpy is shown as a thin line for comparison. The results shown in Figure 7 suggest that the mechanism of stabilization of tRNA^{Phe} structure by polyamines differs from polyamine to polyamine, because the pattern of the decomposed peaks was unique for each polyamine. Especially, decomposed thermogram for tetrakis(3-aminopropyl)ammonium (Figure 7H) differs remarkably from those in the presence of other polyamines.

Despite the fact that tetrakis(3-aminopropyl)ammonium was the most effective polyamine for increasing the apparent melting temperature of tRNA^{Phe} (Table 3), it did not increase the change in enthalpy associated with the thermal melting of tRNA^{Phe}. Figure 8 shows the temperature of the change in enthalpy of melting of tRNA^{Phe} in the presence of various polyamines. The changes in enthalpy correlate with the apparent T_m except for the thermal denaturation in the presence of tetrakis(3-aminopropyl)ammonium.

Table 3 Summary of the thermal denaturation of tRNA^{Phe} in the presence of various polyamines at a concentration of 0.2 mM

ΔH_{cal} is the total enthalpy change of melting, calculated by summing the enthalpies for each of the simulated component DSC peaks. ΔH_{cal} were averaged for at least three experiments. T_m values were averaged for at least three experiments and the average errors in these values were ± 0.1 °C.

Polyamines	Peak	T_m (°C)	ΔH_{cal} (kJ/mol)	Total ΔH_{cal} (kJ/mol)
None	1	40.7	33.3	724.9
	2	47.0	332.2	
	3	56.0	114.6	
	4	68.7	244.8	
Mg ²⁺	1	45.2	21.4	1126.4
	2	56.3	690.4	
	3	66.2	276.1	
	4	76.1	138.5	
Thermine 333	1	43.7	67.4	1246.5
	2	57.5	20.2	
	3	61.8	723.8	
	4	70.7	435.1	
Spermine 343	1	32.1	177.8	1516.7
	2	45.5	117.2	
	3	62.5	648.5	
	4	74.3	573.2	
Caldopentamine 3333	1	36.0	197.1	1535.6
	2	62.7	151.0	
	3	67.6	899.6	
	4	78.5	287.9	
Caldohexamine 33333	1	54.2	338.5	1788.7
	2	72.7	328.9	
	3	77.0	636.0	
	4	81.0	485.3	
Mitsubishine 3(3)3	1	49.2	21.6	1190.2
	2	60.4	866.1	
	3	69.6	63.2	
	4	74.5	239.3	
Tetrakis(3-aminopropyl)ammonium 3(3)(3)3	1	55.7	224.3	737.3
	2	65.0	202.9	
	3	74.4	254.4	
	4	84.1	55.7	

DISCUSSION

Polyamines have been shown to bind to nucleic acids, leading to stabilization of their structure or changes in their conformation [13,16]. Most organisms, including both prokaryotes and eukaryotes, produce only three standard polyamines. In contrast, extreme thermophiles generally produce two additional types of polyamine: longer linear polyamines and/or branched polyamines. To clarify the physiological role of these unusual polyamines, we analysed their effects on the thermal denaturation temperatures of synthetic DNAs and a single molecular species of tRNA. The thermal melting profiles and enthalpy changes associated with melting of the test nucleic acids were recorded using DSC.

Longer polyamines have been shown to stabilize dsDNA more effectively than shorter polyamines [1,13,14]. This conclusion was confirmed in the present study, in which we compared the effects of longer polyamines with those of standard polyamines in a more systematic fashion. The change in enthalpy associated with the thermal denaturation increased proportionally with the melting temperature, suggesting that the mechanism of stabilization of dsDNA by distinct polyamines was similar and independent of the chemical structure of any particular polyamine. Linear polyamines containing only propyl groups within their carbon backbone led to increases in the melting temperature of dsDNA, which were proportional to the number of nitrogen atoms in the individual polyamines, suggesting that charge–charge or

Figure 7 Decomposition of the observed melting curves of tRNA^{Phe}

Experiments were conducted in the absence (A) and in the presence of Mg²⁺ (B), thermine (C), spermine (D), caldopentamine (E), caldoheptamine (F), mitsubishine (G), tetrakis(3-aminopropyl)ammonium (H). In each plot, the dark lines represent the minimum number of non-two-state component transitions required to fit the experimental curve. The thin line is the sum of the component transition peaks.

Figure 8 Temperature dependence of the enthalpy change of melting of tRNA^{Phe}

Experiments were conducted in the absence (A) and in the presence of Mg²⁺ (B), thermine (C), spermine (D), caldopentamine (E), caldoheptamine (F), mitsubishine (G), tetrakis(3-aminopropyl)ammonium (H).

charge–dipole interactions contribute significantly to the stabilizing effect of polyamines on nucleic acids. The longer polyamines, which are often found in extreme thermophiles as major components, stabilize DNAs more effectively than standard polyamines.

When we compared the effects of a polyamine consisting of propyl groups alone with its homologue (in which a propyl group is replaced with a butyl group), we found that the homologue raises the T_m of the test DNA by 0.9°C relative to the

polyamine consisting of propyl groups alone. By way of example, homocaldopentamine stabilized dsDNA more effectively than caldopentamine, despite the fact that both polyamines contained two amino and three aza nitrogen atoms. It is interesting to note that the distance between two nitrogen atoms separated by a butyl group in a polyamine is approx. 5 Å, which corresponds well with the distance between the phosphate groups of adjacent nucleotides in dsDNA.

Unlike dsDNA, thermal denaturation of tRNA is not an all-or-none reaction, but consists of multiple steps. Computational analyses have shown that the thermal denaturation of yeast tRNA^{Phe} could be decomposed into at least five steps. The pattern of decomposition of the thermal denaturation differed for each polyamine, suggesting that distinct changes in the conformation of the tRNA were induced by different polyamines. Nevertheless, the CD spectrum of the tRNA did not change significantly in response to the polyamines, suggesting that they induced only minor conformational change(s) and no change in the secondary structure. Since the change in the pattern of the decomposed thermogram by the addition of a polyamine is so drastic, it is impossible to assign the rise of T_m of each peak shown in Figure 7(A) to the tRNA's three-dimensional structure.

Longer linear polyamines increased the T_m of tRNA more effectively than shorter ones, as was the case with dsDNA. A particularly interesting observation in the present study was the strong positive effect of a branched polyamine, tetrakis(3-aminopropyl)ammonium, on the T_m of tRNA. The decomposed thermogram of tRNA in the presence of tetrakis(3-aminopropyl)ammonium is shown in Figure 7(H). In contrast with the effects of polyamines on dsDNA, the total enthalpy change associated with the thermal denaturation of tRNA was not proportional to the apparent melting temperature. Particularly, the change in enthalpy in the presence of tetrakis(3-aminopropyl)ammonium was significantly smaller than those observed in the presence of other polyamines.

As shown in Figure 8, although the change in enthalpy on thermal melting of tRNA was proportional to the apparent melting temperature, the change in enthalpy in the presence of tetrakis(3-aminopropyl)ammonium did not obey this empirical rule, suggesting that tetrakis(3-aminopropyl)ammonium induced a change in the conformation of tRNA, which was distinct from that induced by other polyamines. We previously found that tetrakis(3-aminopropyl)ammonium stimulated *in vitro* protein synthesis in cell-free extracts of *T. thermophilus* more effectively than other polyamines [12], and that it alone among polyamines was capable of inhibiting Phe-tRNA^{Phe} formation [32]. The inhibition of Phe-tRNA^{Phe} formation by tetrakis(3-aminopropyl)ammonium was counteracted by the addition of any linear polyamine to the reaction. It is thus interesting that branched quaternary polyamines are the major cellular polyamines in some hyperthermophiles such as *Aquifex pyrophilus* and *Methanococcus jannaschii* [33].

The S1 deoxyoligonucleotide used in the present study forms a stem-and-loop structure that denatures in a two-state manner on heating. Similar to what was found for tRNA, S1 ssDNA was more effectively stabilized by tetrakis(3-aminopropyl)ammonium when compared with other polyamines tested. More detailed comparison of the effect of polyamine on ssDNAs with different length of stem is a subject of our future study.

In summary, we conclude that long linear polyamines such as caldopentamine or caldoheptamine stabilize dsDNA and stem parts of RNA effectively, and tetrakis(3-aminopropyl)ammonium is more effective in stabilizing RNAs' stem-and-loop structures. It seems that the two different categories of unique polyamines produced by *T. thermophilus* play two distinct roles in stabilizing

nucleic acids *in vivo*. Longer polyamines can bend to form various shapes. For instance, they can form an L-shaped or S-shaped structure, whereas branched polyamines cannot. Thus, longer polyamines are more flexible and thus better capable of interacting with both strands of DNA across the major or minor groove and at the same time interact with bases inside the double helix. This speculation is supported by X-ray crystallographic studies that have shown that one terminal nitrogen atom of spermine interacts with a phosphate group in one strand of dsDNA and that the terminal nitrogen at the other end interacts with a phosphate on the other strand of DNA. The spermine molecule crosses the major groove, and nitrogen atoms within the central part of the molecule form hydrogen bonds with -OH or -NH groups of the bases of the nucleic acid [24]. dsDNA and stem regions of tRNA are structurally similar to each other, although the former is in a B-form and the latter is in an A-form. Thus, interactions similar to those found between dsDNA and spermine might also form between stem parts of RNA and spermine or other linear polyamines.

In contrast with long linear polyamines, which are string-like molecules with positive charges along their entire length, tetrakis(3-aminopropyl)ammonium comprises four aminopropyl groups projecting outwardly from a central nitrogen atom with positive charges on its surface. A single strand of nucleic acid, such as the loop regions of tRNA might be able to wind around a molecule of tetrakis(3-aminopropyl)ammonium. It might be possible that a tetrakis(3-aminopropyl)ammonium molecule interacts with the phosphate groups within the loop region of a stem-and-loop structure, and at the same time with other phosphate group(s) within the stem region, such that it acts as a bridge connecting phosphate groups on a loop and/or stem regions, thereby strengthening the overall tRNA structure. To examine this possibility directly, we are currently investigating the structure of tRNA-tetrakis(3-aminopropyl)ammonium complexes using NMR imaging.

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