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Interactions between natural polyamines and tRNA: An ^{15}N NMR analysis

(spermine/spermidine/binding to tRNA/hydrogen bonding)

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ABSTRACT **^{15}N NMR spectroscopy was used to explore the interactions between natural polyamines and *Escherichia coli* tRNA. It was found that when tRNA is added to solutions of ^{15}N-labeled spermine or spermidine, there is a considerable decrease in the relative heights of the —NH_2^+— resonances with respect to the signals arising from the —NH_3^+ groups. The presence of tRNA was also found to reduce the longitudinal relaxation times T_1 of the nitrogens, mainly those of the —NH_2^+— groups. The longitudinal relaxation times of the nitrogens were used to characterize the temperature dependence of the binding, and they allowed us to calculate the activation energies that determine the correlation times of amino groups in the presence of tRNA. Both the thermodynamic and the relaxation results indicate that (*i*) spermine binds more strongly to tRNA than spermidine does and (*ii*) within each of these molecules the —NH_2^+— groups bind more strongly to tRNA than the more electropositive —NH_3^+ moieties. This specificity suggests that the interaction between polyamines and tRNA cannot be described exclusively in terms of electrostatic forces and that other interactions (most likely, hydrogen bonding) are very important for establishing the polyamine–tRNA link. Some of the factors that may conspire against the binding of —NH_3^+ groups to tRNA are briefly discussed.**

Putrescine, spermidine, and spermine are natural polyamines widely distributed in biological systems, which under physiological conditions exist in the polycationic forms shown in Fig. 1. Because of their charged character these small molecules interact with DNA and RNA, inducing a stabilization in the tertiary structures of these nucleic acids and exerting a considerable effect on the synthesis of proteins (1, 2). Different experiments have been carried out to explore the nature of the polyamine–nucleic acid interaction. According to the models proposed by Tsuboi (3) and by Liquori *et al.* (4), the charged nitrogens of spermidine and spermine interact with the phosphate groups of the nucleic acid structures by bridging the narrow groove of the double helix. This postulate, however, could not be completely verified by the x-ray analysis of the tRNA–spermine complex, which shows two polyamine molecules bound to the nucleic acid: one in the major groove and the other between two nonhelical strands (5). Two or three strongly bound polyamine molecules were also found by Cohen and his co-workers in studies on tRNA (6), which revealed the presence of 14 or 15 other relatively weak binding sites for spermidine. When the tRNA–polyamine binding was explored by equilibrium dialysis, approximately 13–14 binding sites were found (7), characterized by apparent dissociation constants ranging between 10^{-3} M and 10^{-6} M, depending on the ionic strength of the system.

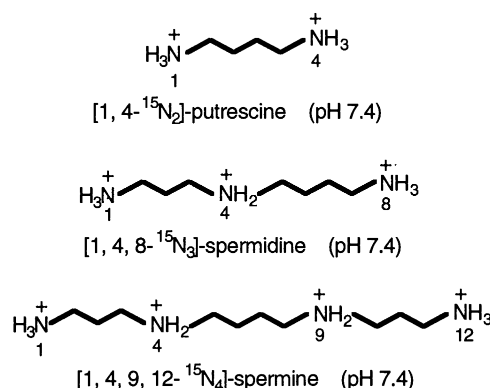

FIG. 1. Structures of the ^{15}N-enriched natural polyamines at pH 7.4, showing the labeling of the nitrogens.

NMR has also been used by several workers to explore these interactions. The power of this method lies not only in the structural details that may arise from the chemical shift values of the observed nuclei but also in the several relaxation phenomena, through which NMR can afford information about the dynamic details of the polyamine–tRNA binding. ^1H NMR, for example, has been used to observe the ways in which polyamines influence the rates of exchange of imino resonances in nucleic acids (8). Wemmer *et al.* (9) also focused ^1H NMR on the protons of spermine when studying the possible interaction between this molecule and a deoxyoligonucleotide duplex, in an analysis that did not reveal the presence of tight binding. Recently, we have exemplified the usefulness of studying polyamine–nucleic acid interactions by observing the NMR spectra of dilute nuclei in isotopically enriched polyamines. *In vivo* ^{13}C NMR showed that putrescine binds to ribosomes but not to the tRNA or DNA fractions of *Escherichia coli* cells (10), whereas spermidine binds strongly to the three macromolecular structures. Subsequent studies allowed us to measure the room-temperature dissociation constants for the spermidine–tRNA equilibrium as a function of the polyamine concentration and of the ionic strength of the sample, and they revealed the presence of two or three strong binding sites in the tRNA backbone even at relatively high Mg^{2+} and K^+ concentrations (11).

One of the main conclusions arising from the spectroscopic as well as from the biochemical analyses is that the interactions between polyamines and tRNA can be classified into two kinds. A stronger binding takes place when the polyamine-to-tRNA ratio is smaller than 4:1, whereas a weaker—and probably less specific—interaction characterizes the binding at higher polyamine-to-tRNA ratios. It is the purpose of the

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present work to discuss the molecular details involved in the binding process of natural polyamines under the first of these two conditions. To carry out this study, ^{15}N NMR spectra of isotopically enriched [1,4,9,12-$^{15}N_4$]spermine and [1,4,8-$^{15}N_3$]spermidine in the presence of *E. coli* mixed tRNA were recorded at polyamine-to-tRNA ratios of ca. 3:1 and concentrations of supporting electrolyte like those *in vivo*. Considerable changes were observed in the single-pulse NMR spectra of a solution of the polyamines upon addition of tRNA, changes that were also evident when the longitudinal relaxation times of the ^{15}N nuclei were compared in the presence and in the absence of the nucleic acid. Variable-temperature analyses carried out on these samples provided the activation energies characterizing the correlation times of the nitrogens in the presence and absence of tRNA. In all these experiments the NMR parameters of —NH_2^+— resonances were more affected by the presence of tRNA than the ones of —NH_3^+ peaks, a fact which suggests the establishment of specific interactions between tRNA and the amino groups of polyamines. For the sake of completeness the analysis was also carried out on [1,4-$^{15}N_2$]putrescine; no interaction between this polyamine and tRNA could be detected.

MATERIALS AND METHODS

Mixed tRNA from *E. coli* strain W was obtained from Sigma. It was dissolved in 50 mM Tris·HCl buffer, pH 7.4/2 mM EDTA/60 mM NH_4Cl and was dialyzed for 2 hr against the same buffer. All solutions were prepared with glass-distilled water and were treated with Chelex (Bio-Rad) prior to their use. The isotopically enriched polyamines were synthesized as described elsewhere (12) and added to the tRNA solution to yield final 3 mM tRNA, 9 mM polyamine, 20 mM Tris·HCl samples. Spectroscopic measurements were also carried out in samples in which the Tris·HCl buffer was replaced by 20 mM sodium phosphate buffer at pH 7.4, 5 mM sodium cacodylate/50 mM KCl buffer at pH 7.0, or 10 mM Tris·HCl buffer at pH 7.4. In all these cases the observed results were equal, within experimental errors, to the ones that could be measured in the 20 mM Tris·HCl buffer.

^{15}N NMR spectra were recorded at 30.4 MHz on a Bruker MSL300 NMR spectrometer. Single-pulse NMR spectra were recorded using ^1H broad-band WALTZ-16 decoupling and nuclear Overhauser enhancement conditions; longitudinal relaxation times were measured by using the saturation-recovery pulse sequence (13). In all the experiments temperatures were regulated by using a Bruker VT100 variable-temperature accessory, and spectra were referenced to $\delta_{(^{15}NH_4)_2SO_4} = 0$ ppm.

RESULTS

Spermine–tRNA Interaction. The room-temperature ^{15}N NMR spectrum of [1,4,9,12-$^{15}N_4$]spermine at pH 7.4 is shown in Fig. 2, spectrum A. The peaks of the nitrogens appear at 24.98 ppm (—NH_2^+—) and 11.75 ppm (—NH_3^+) downfield from the $(^{15}NH_4)_2SO_4$ resonance, the intensity of the secondary nitrogens being slightly larger due to the longer longitudinal relaxation times T_1 of the fast-rotating —NH_3^+ groups. Upon addition of tRNA to the polyamine solution there is a slight variation in the chemical shifts of the —NH_2^+— (24.40 ppm) and —NH_3^+ (11.85 ppm) groups (Fig. 2, spectrum B), and a considerable change in the relative heights of the peaks. We ascribe these effects to the binding of spermine molecules to tRNA. In principle there are various mechanisms through which this interaction might affect the peak intensities in the spermine spectrum: by changing the longitudinal relaxation times of the ^{15}N, by changing the line widths of the ^{15}N resonances, by changing the degree of nuclear Overhauser

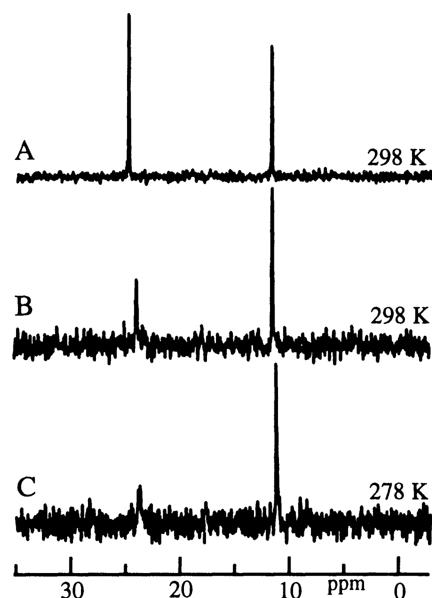

FIG. 2. Spectrum A, ^{15}N NMR spectrum of free [1,4,9,12-$^{15}N_4$]spermine (4 scans). Spectra B and C, ^{15}N NMR spectra of [1,4,9,12-$^{15}N_4$]spermine in the presence of tRNA (100 scans). Spectra were collected at the temperatures indicated to the right of each trace, using 10-μs 90° irradiation pulses and 10-s recycling delays. The ^{15}N NMR spectrum of free spermine shows no changes with temperature.

enhancement affecting each ^{15}N, or by a combination of all of these three factors. In any case, the fact that the binding introduces different changes in the NMR parameters of the two nitrogens suggests a specificity in the tRNA–spermine interaction.

To gain further insight into the details of the binding, ^{15}N spin-lattice relaxation times of free and bound spermine were determined. We found that spermine nitrogens relax faster in the presence of tRNA, implying that upon binding their mean correlation times have increased. Analysis of the spectra indicates that upon binding the T_1 of the —NH_2^+— nitrogens decreases from 3.5 ± 0.2 s to 0.4 ± 0.02 s, whereas the T_1 of the —NH_3^+ groups changes from 7.0 ± 0.4 s in the absence of tRNA to 1.3 ± 0.1 s upon addition of the latter. As we discuss below, these T_1 values place the correlation time of bound spermine at ca. 10^{-10} s, a time scale for which at 7 tesla there should have been almost no variation in the nuclear Overhauser enhancement of the nitrogens. On the other hand, the changes observed in the T_1 values predict that upon addition of the tRNA there will be a decrease in the spin–spin relaxation times, T_2, of the nitrogens, a fact that explains the changes observed in the relative line widths (i.e., in the heights) of the —NH_2^+— and —NH_3^+ resonances that are shown in Fig. 2.

The relative heights of the spermine peaks exhibit a strong temperature dependence, reflecting changes in the rotational dynamics of the amino groups in the presence of tRNA (Fig. 2, spectra B and C). To characterize these changes the longitudinal relaxation times T_1 of the nitrogens of bound spermine were measured at different temperatures; the results of these measurements are summarized in Table 1. Since even in the presence of tRNA spermine molecules are tumbling in the extreme-narrowing regime, these relaxation times are inversely proportional to the correlation times τ_c at each site. Therefore, assuming that the correlation times obey an Arrhenius-type dependence

$$\tau_c = \tau_o \cdot \exp(E_a/RT), \quad [1]$$

Table 1. Longitudinal relaxation times, T_1, of ^{15}N sites in spermine and spermidine in the absence and presence of tRNA, as a function of temperature, T

	T_1, s									
	Spermine				Spermidine					
	Without tRNA		With tRNA		Without tRNA			With tRNA		
T, K	—NH$_2^+$—	—NH$_3^+$	—NH$_2^+$—	—NH$_3^+$	—NH$_2^+$—	—N1H$_3^+$	—N8H$_3^+$	—NH$_2^+$—	—N1H$_3^+$	—N8H$_3^+$
278	2.8	4.3	0.1	0.9	3.2	4.4	5.5	0.7	1.2	1.4
283	3.0	5.0	0.2	1.0	3.7	4.6	5.9	0.9	1.4	1.6
288	3.1	5.0	0.3	1.1	4.0	4.4	6.6	1.0	1.6	1.7
293	3.5	6.5	0.4	1.2	4.7	5.3	7.0	1.1	1.8	1.8
298	3.3	7.0	0.5	1.3	5.0	5.7	7.5	1.2	1.9	2.5
303	3.8	7.4	0.9	1.8	5.4	6.1	8.0	2.2	2.3	2.9

T_1 was measured by using the saturation-recovery sequence, with an accuracy of ca. ±5%.

in which τ_0 is a fixed parameter and R is the gas constant, it is possible to obtain approximate values for the activation energies E_a of the molecular motions from the variation of T_1 with thermodynamic temperature T as

$$\ln(T_1) = A - E_a/RT. \quad [2]$$

Fig. 3 presents the Arrhenius plots that this equation yields for T_1 data of spermine in the presence and absence of tRNA. Again, the different changes observed upon addition of tRNA in the —NH$_2^+$— and —NH$_3^+$ relaxation times constitute strong evidence about the presence of specific spermine–tRNA interactions.

Spermidine–tRNA Interaction. To study the interaction between spermidine molecules and tRNA and to compare it with the results obtained for spermine, an ^{15}N NMR analysis on the binding of [1,4,8-^{15}N$_3$]spermidine was carried out. Fig. 4, spectrum A, shows the spectrum of the free molecule. The frequency of the —NH$_2^+$— peak appears very close to the value observed for the secondary nitrogens of spermine (24.92 ppm), whereas the —NH$_3^+$ nitrogens yield two peaks at $\delta_{[\text{—N1H}_3^+]}$ = 12.04 and $\delta_{[\text{—N8H}_3^+]}$ = 11.72 ppm. As is the case with spermine, addition of tRNA to a spermidine solution produces relatively small changes in the chemical shifts of these ^{15}N resonances (24.24, 11.96, and 11.73 ppm respectively) but considerable changes in the relative heights of the signals (Fig. 4, spectrum B). Moreover, in the case of spermidine these changes are specific, since they affect the —NH$_2^+$— resonances more strongly than the —NH$_3^+$ resonances.

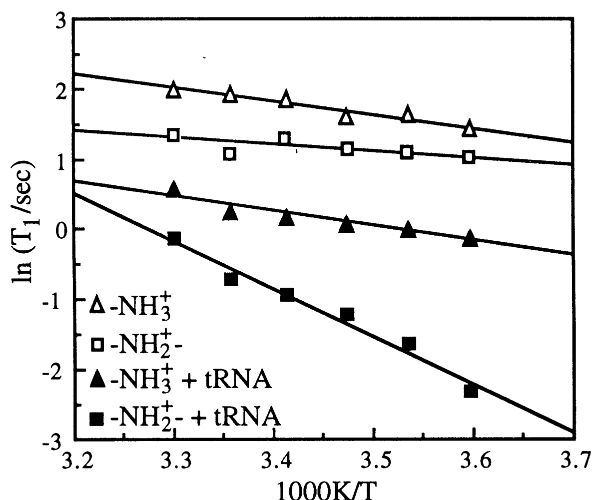

FIG. 3. Natural logarithms of the relaxation times T_1 of —NH$_3^+$ (▲, △) and —NH$_2^+$— (■, □) resonances of spermine in the presence and absence of tRNA, as a function of the inverse of absolute temperature. Lines show the best fit of the experimental data.

This specificity in the spermidine–tRNA interaction is also evident when the rates of spin-lattice relaxation of the nitrogens in free and bound molecules are compared. Whereas in the former case the T_1 of the —NH$_2^+$— is 5.0 ± 0.3 s and the T_1 values of the —N1H$_3^+$ and —N8H$_3^+$ resonances are 5.7 ± 0.3 s and 7.5 ± 0.4 s, respectively, these times shorten to 1.2 ± 0.1 s, 1.9 ± 0.1 s, and 2.5 ± 0.1 s as the molecules bind to tRNA. The behavior of the polyamine signals with temperatures is reminiscent of the variations observed for spermine: the heights of both —NH$_2^+$— and —NH$_3^+$ resonances decrease as the temperature is lowered, the former signal being the most affected (Fig. 4, spectra B and C). These changes were quantified by measuring the relaxation times T_1 of spermidine nitrogens in the presence and absence of tRNA at different temperatures; the results of these observations are summarized in Table 1. As in the case of spermine, these data were interpreted assuming that the relaxation times follow an Arrhenius-type dependence, yielding the plots shown in Fig. 5. Again, these plots reveal different changes in the activation energies of the two kinds of amino groups upon addition of the nucleic acid.

Putrescine–tRNA Interaction. To complete the present study on the binding between natural polyamines and tRNA, ^{15}N NMR spectra of [1,4-^{15}N$_2$]putrescine were obtained in the absence and in the presence of the nucleic acid. In both

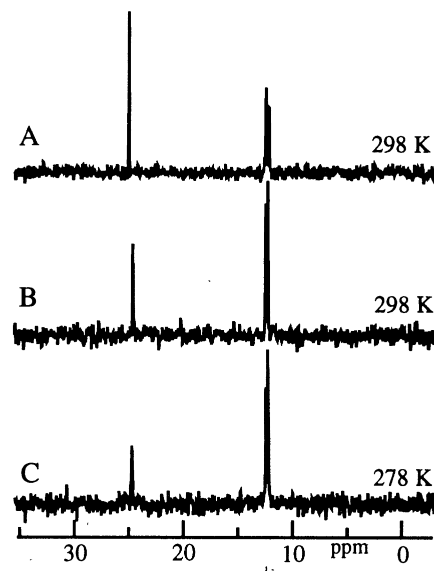

FIG. 4. Spectrum A, ^{15}N NMR spectrum of free [1,4,8-^{15}N$_3$]spermidine (4 scans). Spectra B and C, variable-temperature ^{15}N NMR spectra of [1,4,8-^{15}N$_3$]spermidine in the presence of tRNA (100 scans). Spectra were collected at the temperatures indicated to the right of each trace, using 10-s recycling delays. The ^{15}N NMR spectrum of free spermidine is not dependent on temperature.

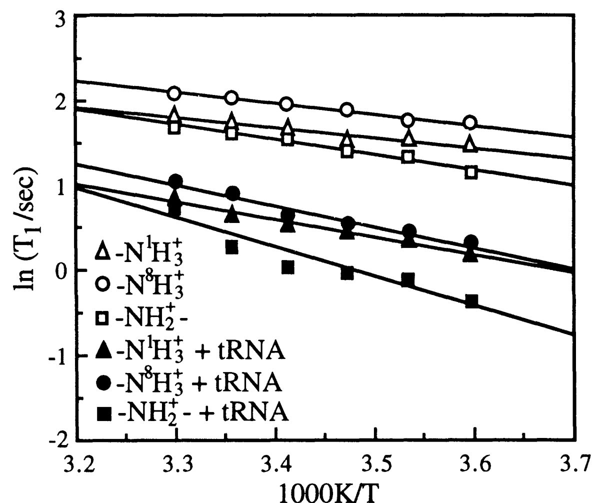

FIG. 5. Natural logarithms of the relaxation times T_1 of —$N1H_3^+$ (▲, △), —$N8H_3^+$ (●, ○), and —NH_2^+— (■, □) resonances of spermidine in the presence and absence of tRNA, as a function of the inverse of absolute temperature. Lines show the best fit of the experimental data.

cases the —NH_3^+ resonance appeared 11.9 ppm downfield from $(^{15}NH_4)_2SO_4$. The lack of secondary nitrogens precludes a "first sight" evaluation of the effects of adding the tRNA. Looking for possible interactions between the putrescine and the nucleic acid, we measured the T_1 of putrescine nitrogens in the absence and in the presence of tRNA. In contrast to what happened with spermine and with spermidine, we found that this T_1 was insensitive to the presence of tRNA (ca. 10.9 ± 1 s), suggesting that putrescine does not interact strongly with the nucleic acid. A similar lack of interaction with tRNA was observed when putrescine was studied in the other buffers listed in *Materials and Methods*.

DISCUSSION AND CONCLUSIONS

The data presented in the preceding section indicate that ^{15}N NMR is a sensitive way to probe the interactions between polyamines and nucleic acids. Before discussing the possible relevance of these data, it is convenient to summarize in numbers the results obtained for the free and the bound polyamines. This is relatively simple for the —NH_2^+— groups, since these moieties do not possess internal motions and their nitrogens are very likely relaxed by the bonded protons. In these cases, relaxation times T_1 can be translated into correlation times τ_c by using the well-known equation for isotropic tumbling valid for the "extreme narrowing" regime in which the reorientation of both spermine and spermidine takes place (13):

$$1/\tau_c = 3.252 \cdot 10^9 \cdot T_1 \cdot n_H, \qquad [3]$$

where $n_H = 2$ is the number of hydrogens directly bound to the ^{15}N (see Table 2). In contrast, interpretation of the relaxation times of primary nitrogens is not as straightforward. Even if nondipolar relaxation pathways (like the spin-rotation mechanism) are disregarded, the presence of a fast rotation along the C—NH_3^+ bond precludes an exact characterization of the dynamics of the molecule at the amino group, and only upper and lower limits can be estimated for the correlation times (14). The results of these assumptions are summarized in Table 2, which also lists the activation energies of the motions obtained in the variable-temperature analyses shown in Figs. 3 and 5. For the purpose of comparison, the results obtained by Komoroski and Allerhand (15) in their study about the motional characterization of tRNA were also included in Table 2.

A meaningful correlation appears when the values obtained for the activation energies and correlation times of —NH_2^+— and —NH_3^+ groups are compared: for each polyamine the largest change in rotational activation energy corresponds to the amino group whose average reorientation time has been most affected by the presence of tRNA. What is not yet clear is the exact molecular processes that these values are reflecting. Given the binding constants for the polyamine–tRNA interaction under the conditions of the present experiments, the concentration of free polyamines should be very small, and yet the correlation times of bound polyamines are considerably shorter than those observed in tRNA. Since the T_1 values (and therefore the correlation times) of tRNA were shown to be practically independent of temperatures below 55°C (15), it is likely that the changes with temperature observed in the ^{15}N NMR parameters of the polyamines are

Table 2. Correlation times and activation energies at the ^{15}N sites of polyamines in the absence and presence of tRNA

System	Group	τ_c,* s	E_a, kcal/mol
Free spermine	—NH_2^+—	$4.4 \cdot 10^{-11}$	1.8 ± 0.1
	—NH_3^+	$2.6 \cdot 10^{-11}$†–$2.9 \cdot 10^{-12}$‡	3.8 ± 0.3
Spermine + tRNA	—NH_2^+—	$3.8 \cdot 10^{-10}$	13.5 ± 1.0
	—NH_3^+	$6.9 \cdot 10^{-10}$†–$7.6 \cdot 10^{-11}$‡	4.2 ± 0.3
Free spermidine	—NH_2^+—	$2.9 \cdot 10^{-11}$	3.5 ± 0.4
	—$N1H_3^+$	$2.0 \cdot 10^{-10}$†–$2.2 \cdot 10^{-11}$‡	2.4 ± 0.3
	—$N8H_3^+$	$1.5 \cdot 10^{-10}$†–$1.7 \cdot 10^{-11}$‡	2.6 ± 0.2
Spermidine + tRNA	—NH_2^+—	$1.2 \cdot 10^{-10}$	6.4 ± 0.7
	—$N1H_3^+$	$6.0 \cdot 10^{-10}$†–$6.7 \cdot 10^{-11}$‡	3.9 ± 0.3
	—$N8H_3^+$	$4.6 \cdot 10^{-10}$†–$5.1 \cdot 10^{-11}$‡	2.8 ± 0.2
Free putrescine or putrescine +tRNA	—NH_3^+	$1.7 \cdot 10^{-11}$†–$1.9 \cdot 10^{-12}$‡	§
tRNA¶	Ribose carbons	$3.3 \cdot 10^{-8}$ or $1.3 \cdot 10^{-9}$‖	≈0

1 kcal = 4.18 kJ.
*Obtained from the T_1 values given in the text by use of Eq. 3 and ref. 14.
†If rapid internal rotation of the —NH_3^+ group is assumed (14).
‡Assuming that the —NH_3^+ group has no internal rotation (14).
§Not measured.
¶From ref. 15.
‖Depending on whether the tumbling of the tRNA molecules takes place in the extreme-narrowing regime.

the result of either (*i*) a binding of polyamines to a site (or sites) whose correlation time is shorter than the mean tRNA reorientation time, and therefore the motion of this site is reflected in the ^{15}N NMR spectra; or (*ii*) an equilibrium process in which polyamines can bind either to fixed sites on tRNA or to very mobile ones [e.g., in the counterion sphere (16)], and therefore the measured activation energies are reflecting a shift in the equilibrium constant of the molecules between these two sites.

Both the relaxation data and the thermodynamic data listed in Table 2 indicate that spermine binds more strongly to tRNA than spermidine does, while the binding of putrescine to tRNA is negligible. Although these three molecules have diaminobutane backbones (Fig.1), the NMR measurements show that their binding strengths are highly dependent on the chemical nature of the nitrogens located in this moiety. In all the cases —NH_3^+ groups bind to tRNA much more weakly than —NH_2^+— groups, suggesting the existence of a factor that precludes the interaction between primary nitrogens and tRNA. This factor is probably not of an electrostatic nature, since primary nitrogens possess a higher density of positive charge than disubstituted amino residues. These observations cast doubts on the simplistic notion that polyamines are just "organic cations," analogous to Mg^{2+} or K^+ but with a hydrocarbon chain. Instead, they suggest that the specificity with which nitrogens in polyamines bind to tRNA is a consequence of the different hydrogen bonding modes that can be established between both types of molecules. There are two factors that could explain the absence of strong hydrogen-bond links between the —NH_3^+ groups of polyamines and tRNA. On one hand, the primary amino residues could fail to stabilize the positive charge on the nitrogen and would allow only the presence of labile N—H bonds, which would break as soon as hydrogen bonds between the —NH_3^+ and the nucleic acid are formed. On the other hand, the high symmetry of —NH_3^+ groups favors the presence of fast diffusive rotations around the C—N axis (similar to the ones that take place in methyl groups), which will hinder the formation of stable N—H···X hydrogen bridges. Further experiments are needed to establish which of these two factors is responsible for the absence of a stable binding between the —NH_3^+ of polyamines and tRNA.

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