Proton exchange rates in transfer RNA as a function of spermidine and magnesium

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ABSTRACT

Solvent exchange rates of selected protons were measured by NMR saturation recovery for *E. coli* tRNA^{Val}, *E. coli* tRNA^{Met} and yeast tRNA^{Phe}, at temperatures from 20 to 40°C, in the presence of 0.12M Na⁺ and various levels of added spermidine. tRNA^{Val} was also studied with added Mg⁺⁺. The exchange rates in zero spermidine and Mg⁺⁺ indicate early melting of the U8 A14 interaction, in accord with thermodynamic melting studies. Exchange rates for secondary protons suggest early melting of the T stem in tRNA^{Met} and the acceptor stem in tRNA^{Phe}, in contradiction with melting transition assignments from thermodynamic work. Addition of 10 spermidines per tRNA stabilizes the secondary and tertiary interactions more effectively than added Na⁺, but less so than Mg⁺⁺. Added spermidine has the curious effect of increasing the exchange rate of the Ψ55 N1 proton, while protecting the Ψ55 N3 proton from exchange in all three tRNA's. Added Mg⁺⁺ has the same effect on tRNA^{Val}.

INTRODUCTION

The thermal melting of tRNA has been extensively studied; in particular, the effects of Na⁺ and Mg⁺⁺ ions on the thermodynamics and kinetics of melting are well documented for several pure species.^{1,2} As a result, some insight has been gained into the roles of these cations in forming and stabilizing tRNA structure. However, despite the recognized importance of polyamine-nucleic acid interactions,³ the study of tRNA melting in the presence of spermidine and spermine has been largely neglected.

To date, two reports have appeared which suggest that Mg⁺⁺ and polyamines have roughly similar effects on the thermal stability of tRNA. Sakai and Cohen³ showed that in the absence of Na⁺, 20 spermidines per tRNA, (corresponding to free spermidine levels near 1 mM)⁴ completely suppressed the melting of mixed *E. coli* tRNA below 50°C, as judged by absorbance at 340 nm. Using NMR, Bolton and Kearns⁵ found that addition of four spermines per tRNA prevented the loss of several ring NH exchangeable proton resonances at 44°C in 0.17 M Na⁺. In the former study, the effects of Mg⁺⁺ were found to be comparable to those of polyamines, as regards the number of cations needed to produce stabilization,

while in the latter, Mg^{++} was found to be somewhat more effective, with spermidine and Mg^{++} together still more effective.

We have studied the effects of low levels of spermidine on the thermal stability of E. coli tRNA^{Val}, E. coli tRNA^{fMet} and yeast tRNA^{Phe}, and the effects of Mg^{++} on E. coli tRNA^{Val}, using NMR saturation recovery⁶ to measure the solvent exchange rates of selected protons resonating in the low field region. Solvent exchange has been widely used to study the conformational dynamics of nucleic acids.⁶⁻⁹ For imino and imido protons which are inaccessible to solvent in the intact nucleic acid structure, the rate limiting step of solvent exchange appears to be an opening, or a disruption, of the structure.⁸ Therefore, the solvent exchange rates are sensitive indicators of local stability, and a rapid increase in exchange rate with increasing temperature is usually diagnostic of the onset of a melting transition involving the protons in question.⁶⁻⁸

The measurements were made in the temperature range 20°-40°C, where the early melting transitions occur. Attempts to study higher temperatures led to irreversible changes in the spectra of the E. coli tRNA's, as discussed later. We have focussed our attention on a small number of resolved resonance whose assignments rest on experiments involving nuclear Overhauser effects (NOE), selective biosynthetic deuteration, biological under-modification, and highly specific photochemical modification. The saturation recovery rates at several temperatures were measured first in 0.12 M Na^+ with no additional cations, and then in the presence of progressively increasing levels of spermidine or Mg^{++} . Details of the relation between the measured saturation recovery rates and the actual solvent exchange rates are given with results below.

MATERIALS AND METHODS

tRNA Samples

Yeast tRNA^{Phe} was purchased from Boehringer Mannheim; E. coli tRNA^{Val} and E. coli tRNA^{fMet} were purified from crude E. coli tRNA (Plenum) as described elsewhere.¹⁰ Amino acid acceptance was assayed with crude yeast or E. coli synthetases. The specific activities, in units of nmol of amino acid per absorbance unit at 258 nm were 1.5 for tRNA^{Phe}, 1.3 to 1.6 for tRNA^{Val} and 1.6 for tRNA^{fMet}.

Samples for NMR, consisting of 5 to 6 mg of tRNA in 0.16 ml of buffer, were prepared by extensive dialysis in a flow microdialysis cell at 4°C. Buffers were prepared in acid washed glassware using glass distilled H₂O and reagent grade chemicals. All dialysis buffers contained 100mM NaCl, 10 mM

Na₂HPO₄, pH 7.0, and varying amounts of Na₂ EDTA, either 20 mM, 10 mM, 1 mM, or zero. Buffers for *E. coli* tRNA also contained 1 mM Na₂S₂O₃. Each change of dialysis was for 12 hours with a volume ratio of 600:1. In the final dialysis, the buffer contained 5% D₂O (Wilmad, 99%) for spectrometer field lock. For dialysis versus zero EDTA, a small column of Chelex was placed in series with the dialysis cell and buffer reservoir.

Samples 1 and 2 (tRNA^{Val}) were dialyzed against two changes each of 10 mM EDTA buffer and 1mM EDTA buffer. Sample 3 (tRNA^{Val}) was dialyzed against two changes each of buffers containing 20 mM EDTA, 10 mM EDTA, and 5mM EDTA, and zero EDTA. Sample 4 (tRNA^{Val}) was similar to samples 1 and 2 except for two additional dialyses versus zero EDTA buffer.

Two samples of tRNA^{fMet} (samples 5 and 6) and a sample of tRNA^{Phe} (sample 7) were all prepared by dialysis against two changes each 20 mM EDTA and 5 mM EDTA buffer.

NMR Spectroscopy

All data were as collected at 270 MHz on the LDB 270 spectrometer at Brandeis University. Saturation recovery rates were measured by the interleaved difference method as described elsewhere.¹⁰ The estimated error in the reported rates is $\pm 15\%$. Temperatures were monitored continuously with a copper constantan thermocouple, and are believed accurate to $\pm 1^\circ\text{C}$. Variation in temperature during a typical run (12 hours) was typically $\pm 0.5^\circ\text{C}$. Saturation pulses were 0.1 sec in length, and the total time between pulse sequences was at least twice the observed relaxation time. Spin diffusion (multiple spin flips) could affect our results but only slightly and in the low temperature regime, where magnetic relaxation dominates.

As far as possible, data with and without added spermidine or Mg⁺⁺ were collected on the same tRNA samples. This was not possible for tRNA^{fMet} due to some irreversible intensity losses in the spectrum after prolonged exposure to high temperatures with no added spermidine (see below). Spermidine and Mg⁺⁺ were added directly to tRNA by pipetting an aliquot of counterion solution (50 mM spermidine in 1 mM EDTA dialysis buffer, 100 mM Mg⁺⁺ in 100 mM NaCl) onto clean parafilm, adding about 0.05 ml of tRNA withdrawn from the NMR tube, and transferring the resulting mixture back into the NMR tube. Transfers were performed with a sterile syringe and an acid washed teflon needle.

Irreversible denaturation of tRNA

We found that the prolonged exposure to high temperatures required in this work (12 to 24 hours at $T > 35^\circ\text{C}$) produced gradual irreversible intensity losses in some resonances of the *E. coli* tRNA's. We believe this is due to

aggregation, encouraged by the high concentrations of tRNA needed for NMR. In some cases the tRNA could be restored, as judged from its spectrum, by heating and slow cooling in the presence of Mg^{++} or spermidine. All of the data reported herein were obtained from tRNA samples which had not suffered noticeable intensity losses. The temperature limits to which the tRNA's could be pushed without damage were empirically established in preliminary experiments, at the cost of several samples. Yeast tRNA^{Phe} does not suffer from the problem described above.

RESULTS AND INTERPRETATION

E. coli tRNA^{Val}

Figure 1A shows the spectrum of tRNA^{Val} (sample 1) at 30°C in the absence of Mg^{++} or spermidine. Peak A was shown¹¹ to be s⁴U8-A14 resonance in a study of undermodified tRNA^{Val}; peak P was assigned to an imino proton of the GU base pair (GU50) by nuclear Overhauser effect (NOE).¹² NOE studies also established that peak Q is the resonance of the Ψ55 N1 proton¹⁰ and O is that of the Ψ55 N3 proton (B. Reid, personal communication.) Peaks O and P are coalesced in the absence of Mg^{++} or spermidine, below 30°C. NOE experiments on purine C8 deuterated tRNA^{Val} (A. Redfield and V. Sánchez, unpublished) have shown that B is due to a Watson Crick AU base pair. It has been assigned to AU6 by interbase-pair NOE (B. Reid, private communication). Saturation recovery data on these five peaks are shown in Tables I and II; data for the large multiproton peak K, and for the partially resolved peaks C and E are not shown. Hare and Reid¹³ recently assigned all the ring NH resonances of the D stem in *E. coli* tRNA^{Val} by NOE at 500 MHz; unfortunately none of these are resolved at 270 MHz.

As discussed elsewhere,⁷ the measured saturation recovery rate is a sum of solvent exchange and magnetic relaxation contributions. The magnetic relaxation rate for most protons in tRNA is dominated by spin diffusion, and is roughly 5 sec⁻¹ independent of temperature. The magnetic rate may actually decrease at elevated temperatures. Therefore, any increase in saturation recovery rate is ascribable to an increase in solvent exchange rate. The data shown herein are uncorrected for magnetic relaxation.

In the absence of spermidine, peaks A and B show sharp rate increases between 32° and 39°C, indicating the onset of melting. The other resolved single proton peaks show small rate increases which might possibly signal the onset of melting or a general loosening of the structure. The recovery rate for the multiproton peak K was constant at about 10 sec⁻¹ between 22° and 39°C;

Figure 1. 270 MHz NMR spectra of the three tRNA's studied, at 20°C, 0.1 M NaCl, 10 mM sodium phosphate pH7, and in the case of *E. coli* tRNA's, 1 mM sodium thiosulphate. A, *E. coli* tRNA^{Val}; B, *E. coli* tRNA^{Met}; C, yeast tRNA^{Phe}.

peaks C and E had rates of roughly 10 sec⁻¹ at 20°C and 25 sec⁻¹ at 39°C.

The rates, particularly for peaks A and B in zero Mg⁺⁺ and spermidine, show some variation from sample to sample. Both peaks appear to melt more rapidly in sample 2 than in sample 1, and more rapidly still in a sample 3. We believe that the variations are due to minor contamination with Mg⁺⁺, since sample 3 was subjected to the most rigorous dialysis (20 mM EDTA). Peak A, s⁴U8-A14, shows the greatest variability, and is therefore presumed most sensitive to Mg⁺⁺. Despite these variations, the general trends and conclusions are the same for all samples, as discussed below.

Progressive addition of spermidine stabilizes the s⁴U8-A14 interaction (peak A) as well as the secondary base pair B (Table I). Added spermidine also

Table I. Recovery rates (sec^{-1}) for *E. coli* tRNA^{Val} vs. spermidine.

Spermidines Added per tRNA(a)	Sample	T°C	PEAK AND ASSIGNMENT				
			A s ⁴ U8-A14	B AU6	O Ψ55N3	P GU50	Q Ψ55N1
0	2	19	12	22	10	10	10
0	1	22	9	15	9	9	12
0	2	29	12	28	11	10	15
0	1	32	17	31	13	13	13
0	2	32	28	43	13	13	18
0	1	36	26	50	18	9	13
0	1	39	50	120	27	17	19
4	1	21	10	13	7		12
4	2	21	10	12	7	7	11
4	2	32	10	17	9	8	23
4	1	33	13	17	10	9	16
4	1	36	13	24	10	7	19
4	1	39	28	46			45
4	2	41	32	35	26	23	67
8	2	19	9	11			25
8	1	21	7	9	8		20
8	1	30	8	14	7		20
8	2	30	9	12			33
8	1	38	17	23			30
8	1	41					48
8	2	41					87

(a) Buffer .1 M NaCl .01 M sodium phosphate, pH 7.0, for this and subsequent tables.

appears to slow the exchange of the GU and Ψ55 N3 resonances and suppresses that of peaks C and E. What is surprising is that added spermidine increases the exchange rate¹ of the Ψ55N1 proton (peak Q). The rate increases very sharply above 38°C. This is quite unexpected, especially in light the apparent stabilization of the Ψ55 N3 proton (peak O) by spermidine. Similar rate increases for the Ψ55 N1 proton are observed upon adding spermidine to *E. coli* tRNA^{fMet} and yeast tRNA^{Phe}, and upon adding Mg⁺⁺ to *E. coli* tRNA^{Val}, and yeast tRNA^{Phe}, as discussed below.

Table II shows selected rate data for tRNA^{Val} in the presence and absence of Mg⁺⁺ and at several temperatures. The data from zero to four Mg⁺⁺ per tRNA are from sample 3; the data for ten Mg⁺⁺ are from sample 4. Data on sample 4 in zero Mg⁺⁺ (not shown) are similar to zero Mg⁺⁺ data for sample 2.

The data show that Mg⁺⁺, like spermidine, stabilizes the s⁴U8-A14 base pair. Like spermidine, Mg⁺⁺ also accelerates the exchange of the Ψ55 N1 proton, while at the same time apparently stabilizing the Ψ55 N3 proton. The high

Table II. Recovery rates (sec^{-1}) for E. coli tRNA^{Val} vs. Mg^{++} .

Mg ⁺⁺ added per tRNA(a)	T°C	PEAK AND ASSIGNMENT				
		A s ⁴ U8-A14	B AU6	O Ψ55N3	P GU50	Q Ψ55N1
0	20	2	14	8	8	9
0	27	22	25	11	11	11
0	32	40	45	11	11	15
0	34	42	58	10	10	16
0	36	57	81	16	12	23
2	21	14	18	8	8	10
2	27	13	16	5	5	10
2	32	21	26	10	10	12
4	21	9	9	8	8	8
4	27	11	14	11	11	15
4	33	17	29	12	11	19
10	20	8	12			17
10	27	7	7			
10	35	6	5	7	7	37
10	37	10	10	8	8	55

(a) Samples 3 and 4.

temperature data at 10 Mg^{++} per tRNA are not entirely clear cut, since peaks O and P coalesce at this Mg^{++} level. However, the rate data for the composite peak show no obvious biphasic recovery behavior, so one may state confidently that no significant rate increase occurs for the Ψ55 N3 proton at the temperatures studied here.

E. coli tRNA^{fMet}

Figure 1B shows the spectrum of E. coli tRNA^{fMet} in the absence of spermidine or Mg^{++} at 20°C. Peak A was assigned to the s⁴U8-A14 tertiary base pair in a careful study of photochemical dethiolation¹⁴; peak L is assigned to the imino proton of U50 in the GU base pair (UG50) by NOE¹² and ¹⁵N labeling¹⁵; peak N is assigned to the N1 proton of residue Ψ55, and also by NOE.¹⁰ Peaks B and C have been shown respectively to arise from a Watson Crick AU base pair and a tertiary base pair, by NOE of a purine C8 deuterated sample compared with unlabeled tRNA (Johnston, Sanchez and Redfield, unpublished). Peak C has been shown to be arise from a uracil N3 proton by means of ¹⁵N labeling¹⁵, showing that this is the T54-A58 resonance and not, for example, that of m⁷G46 (R. Griffey, private communication). E. coli tRNA^{fMet} contains only two AU base pairs, AU11 and UA27, but it is not known which pair corresponds to peak B.

Table III. Recovery rates (sec⁻¹) for *E. coli* tRNA^{fMet} vs. spermidine.

Spermidines added per tRNA ^(a)	T°C	PEAK AND ASSIGNMENT					
		A s ⁴ U8-A14	B UA11 or UA27	C T54-A58	L U50	M 55N3	N 55N1
0	22	11	9	9	8	9	11
0	27	14	8	7	11	12	16
0	32	20	9	12	11	15	20
0	38	57	35	33	36	64	51
5	19	13	15	12	12	12	19
5	27	9	7	6	5	9	17
5	32	13	8	7	8	13	29
10	20	7	4	8	9	7	28
10	27	10	7	7	9	9	36
10	35	11	5	9	9	13	102
10	38	25	8	13			

(a) Samples 5 and 6.

(b) Multiple peak probably containing 55N3.

Finally, it is almost certain that the two proton peak M near 11.5 ppm contains the Ψ 55 N3 proton since this proton has been shown by NOE to resonate near 11.5 ppm in *E. coli* tRNA^{Val} (B. Reid, personal communication) and in yeast tRNA^{Phe}, tRNA^{Val} and tRNA^{Asp} (S. Roy and A. Redfield, unpublished).

Table III gives selected saturation recovery data for tRNA^{fMet} at various levels of spermidine. The zero spermidine data are from sample 5; the other data are from sample 6. Data at higher temperatures would be desirable, but the problem of irreversible spectral changes at high temperatures was particularly severe for tRNA^{fMet}. In the absence of spermidine, peaks A, M, and N show marked rate increases at 38°C; peaks B, C, and L show smaller increases. The multiproton peaks F and K (not included in table) show rate increases from roughly 10 sec⁻¹ near at 22°C to about 20 sec⁻¹ at 38°C; multiple recovery times were not discernible.

As with tRNA^{Val}, the rate data for tRNA^{fMet} indicate destabilization of the s⁴U8-A14 interaction in the absence of spermidine, at 38°C. The large rate increases for the Ψ 55 N1 and N3 protons, and the moderate increases for the GU resonance suggest an overall destabilization of the T stem and T loop as well. The rate increase for the AU resonance B may indicate the onset of melting in the D stem and perhaps some fraying of the anticodon stem.

The addition of spermidine produces effects similar to those observed for

Table IV. Saturation Recovery rates (sec^{-1}) for yeast tRNA^{Phe} vs. spermidine.

Spermidines added per tRNA ^(a)	T°C	PEAK AND ASSIGNMENT				
		A UA6	B U8-A14	L GU4	M Ψ55N3	N Ψ55N1
0	20	14	13	15	11	12
0	27	17	15	14	17	18
0	33	22	24	12	23	32
0	36	72	58	42	75	75
5	29	8	8	9	10	15
5	28	15	13	17	13	23
5	36	35	25	28	22	83
10	20	14	11	11	8	29
10	30	17	16	27	14	55
10	36	33	32	42	32	120

(a) Sample 7.

tRNA^{Val}. Most protons are stabilized against solvent exchange at higher temperatures, with the exception of the Ψ55 N1 proton, the exchange rate of which increases dramatically.

Yeast tRNA^{Phe}

Figure 1C shows the spectrum of yeast tRNA^{Phe} (sample 7) in the absence of spermidine or Mg^{++} at 20°C. Five single proton resonances have been assigned by NOE, using purine C8 deuterated tRNA in some cases. Peak A is from the secondary base pair¹⁶ UA6; peak B is U8-A14 tertiary base pair resonance¹⁶; peak L is an imino proton¹² from base pair GU4; peak M is the N3 proton of residue¹⁶ Ψ55; (S. Roy, unpublished) and peak N the N1 proton¹⁰ of Ψ55. Table IV gives the saturation recovery rate data for sample 7. The rates in the absence of spermidine are similar to those observed in earlier work and are discussed in detail elsewhere^{6,7,16}; we include them here mainly for convenient reference.

Addition of five spermidines per tRNA produces effects similar to those observed in the E. coli tRNA's we have studied. The rate increases at high temperature are suppressed for most resonances except that of the Ψ55 N1 proton, the exchange of which is perhaps slightly accelerated. However, at ten spermidines per tRNA, an apparent anomaly is observed. Not only is the Ψ55 N1 proton exchange speeded up, as with the other tRNA's, but several other peaks, B, L and M, appear to exchange more rapidly than at five spermidines per tRNA. The observation of this nonmonotonic behavior is not corroborated

with similar data from other tRNA's and should be viewed with caution. The best we can say is that the effect is probably not an artifact of gradual denaturation, since yeast tRNA^{Phe}, unlike the other tRNA's in this study, does not suffer irreversible spectral changes from prolonged exposure to high temperatures.

DISCUSSION

tRNA melting with no Mg⁺⁺ or spermidine.

It is well known that both the secondary and tertiary structures of most tRNA's are intact in the absence of Mg⁺⁺ or spermidine, at Na⁺ concentrations around 0.1M and temperatures below about 20°C.^{1,2} Extensive thermodynamic studies have been interpreted to show that the structure unfolds in a series of partially resolved transitions (5 for class I tRNA's) between 20 and 100°C. The earliest melting transition is usually assigned to tertiary structure; the other four to the melting of the helical arms; considerable overlap of secondary and tertiary transitions often occurs.^{1,2}

The methods commonly employed in thermodynamic work, i.e. calorimetry, temperature jump, and differential UV spectroscopy, provide limited microscopic details of the melting process. NMR, on the other hand, is capable of giving the full microscopic picture⁶⁻⁸ and should eventually lead to definitive assignments of the thermodynamically detected melting transitions. In what follows we review our melting data in the light of thermodynamic results. In some cases our conclusions agree with the published interpretations of thermodynamic studies; in others, they do not. Some disagreements may arise from uncertainties in correlating individual proton exchange rates with the true degree of thermodynamic melting⁷; others may indicate that the usual classifications of melting transitions into such neat categories as tertiary or D stem, are in fact, oversimplified.

E. coli tRNA^{Val}. From the data on peak A we conclude that the s⁴U8-A14 interaction undergoes an early melting transition. The secondary base pair AU6 represented by peak B also melts early. Small rate increases for Ψ55 and GU resonances may mark the beginning of T loop and perhaps T stem disruption as well, but no firm conclusions concerning T arm melting should be drawn until data at higher temperatures become available. The GU rate increase at 39°C is on the edge of what we consider significant; comparing it to other rate increases in tRNA^{Val}, one might infer that GU base pairs are not inherently unstable.

The melting of E. Coli tRNA^{Val} has not been widely studied; thermodynamic

data are not available. Two previous studies of melting in zero Mg^{++} were performed at very different Na^+ concentrations from ours. Using absorbance at 340 nm, Urbanke *et al.*,¹⁷ found an early melting transition with midpoint at 30°C in 0.02M Na^+ , and assigned it to tertiary melting. Since s^4U8 absorbs at 340 nm, this is clearly consistent with our results. Kastrop and Schmidt¹⁸ monitored the methyl and methylene resonance positions as functions of temperature in 0.23M Na^+ . The earliest transition they found is centered at 55°C, and assigned to the D stem melting. While this appears to contradict our findings, the large difference in salt concentrations probably precludes meaningful comparison.

E. coli tRNA^{fMet}. The melting of this tRNA has been studied by temperature jump,^{19,20} scanning calorimetry,² and NMR, including limited saturation recovery data.^{7,19} The early melting transition has its midpoint at 46°C in 0.15 M Na^+ and has been assigned to the tertiary structure and D stem arm melting.^{2,19} The remainder of the structure melts with a midpoint of 64°C. While our data confirm the early melting of the s^4U8 -A14 tertiary base pair, the rate increases we observe for the GU, T54-A58, $\Psi55$ N1 and possibly the $\Psi55$ N3 resonances suggest a parallel and comparable destabilization of the T loop and T stem, possibly involving disruption of the $\Psi55$ P58 H bond.²¹ The secondary AU base pair resonance B shows rate increases comparable to or smaller than the T arm resonances; this observation suggests that re-examination of the melting transition assignments may be desirable, since it would imply that the T stem melts before the D stem. However, more NMR rate data are needed. A rough comparison of tertiary structure melting rates obtained by NMR with those from temperature jump is given elsewhere.⁷

Yeast tRNA^{Phe}. The melting of yeast tRNA^{Phe} in zero Mg^{++} has been studied by calorimetry,^{2,22} by optical spectroscopy,^{15,17,23,24} and by NMR, including saturation recovery.^{6,7} Our observation of sharp rate increases for the GU4 and AU6 protons supports the notion that the acceptor stem melts early as suggested by Johnston and Redfield.⁷ This disagrees with the assignment of the earliest secondary transition to the D stem, which was made by Privalov and Filimonov by comparing predicted and observed melting enthalpies.²

Our data also indicate early disruption of the T loop structure, as evidenced by rapid exchange of the $\Psi55$ N1 and N3 protons. It is not clear at present whether this behavior could be subsumed under the heading of 'tertiary melting' or whether it marks a beginning of an independent disintegration of the entire T stem structure.

Stabilization of tertiary structure by spermidine.

Na^+ , Mg^{++} , and spermidine all stabilize the tertiary structure of tRNA; we wish to make a rough assessment of the stabilizing power of spermidine relative to that of Na^+ and Mg^{++} . Our reasoning will be semiquantitative at best, with no claim to thermodynamic rigor.

Calorimetric data show that Mg^{++} is a much better stabilizer than Na^+ . Starting from sufficient free Na^+ to form the tertiary structure (about 20 mM at 20°C), added Na^+ increases the assumed tertiary transition midpoint temperature (T_m) by about 20°C per decade of concentration.^{1,2} In contrast, raising the concentration of free Mg^{++} from near zero to 1 mM in the presence of 150 mM free Na^+ increases the assumed tertiary T_m by 20° to 30°C for many tRNA's.²

Thermodynamic melting data for tRNA in the presence of Na^+ and spermidine are not available, and our rate exchange rate data cannot yield definitive values of the shift in T_m with added spermidine. However, one can make rough estimates of the shift, and therefore of the relative gain in stability, by assuming that equality of exchange rates at different temperatures and spermidine levels implies an approximately equal degree of melting. Thus, from Table I, sample 1, the recovery rate for the U8 A14 resonance of tRNA^{Val} is 17 sec⁻¹ at 32° and zero spermidine, and 17 sec⁻¹ at 38°C and eight spermidines per tRNA. This implies a shift in T_m of 6°C for a nominal addition of 24 positive charges per tRNA. Although the free spermidine concentration is not known, one can calculate the shift in T_m that would arise from an increment in free Na^+ concentration of 24 charges per tRNA. Assuming, as above, that $dT_m/d\log[\text{Na}^+] = 20^\circ\text{C}$, the increase in T_m would be 1.7°C. Even within the uncertainty of our measurements and the roughness of the estimate, it would appear that, charge for charge, added spermidine is more effective than Na^+ in stabilizing the U8 A14 interaction in E. coli tRNA^{Val}. Similar conclusions can be drawn for E. coli tRNA^{fMet}. For the U8 A14 interaction in yeast tRNA^{Phe} (peak B) the estimated increase in T_m for five spermidines per tRNA is 3°C, which is larger than expected for the charge equivalent of Na^+ . However, the data for tRNA^{Phe} display an anomaly in that increasing from five to ten spermidines per tRNA appears to produce a small destabilization, but whether this effect is real or artificial remains to be seen.

On the whole, we feel safe in concluding that spermidine is generally more effective than Na^+ in stabilizing tertiary structure. Also, from comparison of Table I with Table II, we conclude that Mg^{++} is more effective than spermidine, in agreement with earlier work.⁵ Thus, in order of stabilizing power, one has $\text{Mg}^{++} > \text{spermidine} > \text{Na}^+$.

The relative stabilizing effects of spermidine and Mg^{++} can also be considered from the point of view of recent work^{25,26} on polyelectrolyte theory applied to nucleic acids: the nucleic acid is surrounded by a Na^+ counterion cloud whose concentration at the surface of the macromolecule is only weakly dependent on the Na^+ concentration in solution, far from the macromolecule, at the concentrations we use. The added spermidine, being triply charged, is even more strongly localized in this counterion layer, and apparently competes successfully with the local high Na^+ concentration for possible binding sites on the tRNA.

Stabilization of secondary structure by spermidine.

It has been argued that melting of the tertiary structure is an obligatory first step in tRNA unfolding,² and that Mg^{++} stabilizes the entire tRNA structure by locking the tertiary structure in place, rather than by acting directly on the helical arms and loops. Employing the reasoning of the previous section to the rate data for early melting secondary resonances, one finds that the apparent shifts in T_m are comparable to the shifts for the U8 A14 tertiary resonances. This is consistent with the view that spermidine stabilizes the secondary structure by locking in the tertiary. On the other hand, both spermidine and Mg^{++} have unusual effects on the solvent exchange behavior of the $\Psi 55$ N1 and N3 resonances, which may indicate that the interaction of tRNA with these cations is more complex than the above-stated view suggests.

Effects of spermidine and Mg^{++} on the $\Psi 55$ N1 proton.

For all three tRNA's studied here, spermidine has the curious effect of increasing the exchange rate of the $\Psi 55$ N1 proton while simultaneously protecting the H bonded N3 proton from exchange. Mg^{++} has a similar effect on E. coli tRNA^{Val}; NOE experiments suggest that the same holds for yeast tRNA^{Phe}.¹⁰ The nature of the structural event associated with this behavior is not clear. Studies of the NOE from the $\Psi 55$ N1 proton to the T54 methyl in all three tRNA's shows no detectable change in local stacking geometry upon going from zero to ten spermidines per tRNA in 0.11 M Na (J. Tropp, unpublished experiments). Earlier NOE work on E. coli tRNA^{Val} and yeast tRNA^{Phe} likewise showed no such effect upon adding Mg^{++} in the presence of 0.11 M Na^+ .¹⁰ Also, no effect on the T54 methyl to G53 C8 proton NOE was observed upon adding Mg^{++} or spermidine to tRNA^{Val} or spermidine to tRNA^{fMet}. Added cations do cause shifts in the $\Psi 55$ N1 resonance position, although not in the NOE-coupled resonances. However, Mg^{++} produces a large (0.1 to 0.2 ppm) upfield shift, while spermidine gives a smaller downfield shift, which suggests that the two cations may act in different ways.

In sum, the available NOE data shows no perturbation, by either cation, of the base stacking in the the T loop and adjacent T helix regions, while chemical shift data indicate unspecified qualitative differences in the action of the two cations. It is conceivable that added cation could directly catalyze proton exchange at the exposed Ψ55N1 position, or do so indirectly by increasing the local hydroxide concentration, promoting hydroxide catalysis.

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REFERENCES

1. Crothers, D. and Cole, P. (1978) in *Transfer RNA* (S. Altman, Ed.) M.I.T. Press, Cambridge, pp. 197-247.
2. Privalov, P. and Filimonov, V. (1978) *J. Mol. Biol.* 122, 447-464.
3. Sakai, T. and Cohen, S. (1976) *Progress in Nucleic Acid Research and Molecular Biology* 17, 15-42.
4. Sakai, T., T'orget, R., I.J., Freda, C., and Cohen, S. (1975) *Nucleic Acids Res.* 2, 1005-1022.
5. Bolton, P. and Kearns, D. (1977) *Biochemistry* 16, 5729-5741.
6. Johnston, P., and Redfield A. (1977) *Nucleic Acids Res.* 4, 3599-3615.
7. Johnston, P. and Redfield, A. (1981) *Biochemistry* 20, 3996-4006.
8. Hurd, R. and Reid, B., (1980) *J. Mol. Biol.* 142, 181-194.
9. Teitelbaum, H. and Englander, S. (1975) *J. Mol. Biol.* 92, 55-78.
10. Tropp, J. and Redfield, A. (1981) *Biochemistry* 20, 2133-2140.
11. Hurd, R. and Reid, B. (1979) *Biochemistry* 18, 4005-4016.
12. Johnston, P. and Redfield A. (1978) *Nucleic Acids Res.* 5, 3913-3913.
13. Hare, D. and Reid, B. (1982) *Biochemistry* 21, 1835-1842.
14. Daniel, W. and Cohn, M. (1975) *Proc. Nat'l Acad. Sci., USA* 72, 2582-2586.
15. Griffey, R., Poulter, C.D., Yamaizumi, Z., Nishimura, S., and Hurd, R. (1982) *J. Amer. Chem. Soc.* 104, 5811.
16. Roy, S. and Redfield, A. (1983) *Biochemistry* (in press).
17. Urbanke, C., Romer, R. and Maass, G. (1973) *Eur. J. Biochem.* 33, 511-516.
18. Kastrop, R. and Schmidt, P. (1975) *Biochemistry* 14, 3612-3618.
19. Stein, A. and Crothers, D. (1976) *Biochemistry* 15, 160-168.
20. Crothers, D., Cole, P., Hilbers, C. and Shulman, R. (1974) *J. Mol. Biol.* 87, 63-88.
21. Rich, A. (1977), *Acc. Chem. Res.* 10, 388-396.
22. Hinz, J., Filimonov, V. and Privalov, P. (1977) *Eur. J. Biochem.* 72, 79-86.
23. Coutts, S., Riesner, D., Romer, R., Rabl, C. and Maass, G. (1975) *Biophys. Chem.* 3, 275-289.
24. Urbanke, C., Romer, R. and Maass, G. (1975) *Eur. J. Biochem.* 55, 439-444.
25. Gueron, M. (1983) in *"Lecture Notes in Physics,"* Springer-Verlag (in press).
26. Anderson, C.F., and Record, M.T. (1983) in *"Structure and Dynamics of Nucleic Acids and Proteins"* (R. Sarma and E. Clementi, eds.), Adenine Press (in press).