Volume 5 Number 1 January 1978 Nucleic Acids Research

^{1}H NMR of valine tRNA modified bases. Evidence for multiple conformations

Rodney V. Kastrup[+] and Paul G. Schmidt*

School of Chemical Sciences, University of Illinois, Urbana, IL 61801, USA

Received 17 November 1977

ABSTRACT

Methyl and methylene protons of dihydrouridine 17 (hU), 6-methyladenosine 37 (m^6A), 7-methylguanosine 46 (m^7G), and ribothymidine 54 (rT) give clearly resolved peaks (220 MHz) for $tRNA_1^{val}$ (coli) solutions in D_2O, 0.25 m NaCl, at 27° C. Chemical shifts are generally consistent with a solution structure of $tRNA_1^{val}$ similar to the crystal structure of $tRNA^{phe}$ (yeast). At least 3 separate transitions are observed as the temperature is raised. The earliest involves disruption of native tertiary structure and formation of intermediate structures in the m^7G and rT regions. A second transition results in a change in structure of the anticodon loop, containing m^6A. The final step involves unfolding of the m^7G and rT intermediates and melting of the TΨC helix. Low salt concentrations produce multiple, partially denatured conformations, rather than a unique form, for $tRNA_1^{val}$. Native structure is almost completely reformed by addition of Na^+ but Mg^{2+} is required for correct conformation in the vicinity of m^7G.

INTRODUCTION

Proton nmr spectra of tRNA offer a detailed look at solution conformation and dynamics of the macromolecule. Most work to date has taken advantage of exchangeable, hydrogen bond resonances observable in H_2O solutions of nucleic acids.[1] Another approach is the use of modified base peaks in the high field region of the proton spectrum.[2,3,4] The latter method has the advantages that peak assignments are independent of assumptions about ring current shifts and helix structure, and that spectra always reflect equilibria of structure.

We report here an nmr investigation of the structure of *E. coli* $tRNA_1^{val}$ using the modified base resonances which occur in the region 4 to 0 ppm. The spectra show evidence of multiple conformations for the TΨC loop, anticodon loop and variable arm, formed during thermal denaturation. The existence of a unique conformation of the molecule at 37° C is shown to be dependent on the presence of sufficiently high counter ion concentration.

MATERIALS AND METHODS

Unfractionated tRNA from Escherichia coli B was purchased from Plenum Scientific Research, Inc. Purified $tRNA_1^{val}$ was isolated using three column steps. The first employed a NaCl gradient on benzoylated DEAE-cellulose.[5] The valine accepting fractions that corresponded to $tRNA_1^{val}$ were pooled, precipitated with ethanol and further purified using a NaCl gradient on DEAE-Sephadex A-50.[6] After valine accepting fractions from this column were pooled and precipitated final purification was achieved using a reverse salt gradient ($(NH_4)_2SO_4$) on a Sepharose 4B column.[7] We thank Dr. Brian Reid, University of California, Riverside, for suggesting the 3 column protocol. After elution from the Sepharose column the $tRNA_1^{val}$ was dialyzed once against 1 M NaCl, 5 mM phosphate, pH 7.0 and three times against 5 mM phosphate, pH 7.0, to remove $(NH_4)_2SO_4$. Material prepared in this way routinely gives amino acid acceptance activities of greater than 1.6 nmoles valine/A_{260} unit.

An nmr spectrum of a sample with this activity was run before the tRNA was further dialyzed to remove Mg (II) ions. At 38° the spectrum of pre-dialyzed $tRNA_1^{val}$ (0.2 M NaCl, 11 mM Mg (II)) was virtually identical to that shown in Fig. 5 for a sample which had been extensively dialyzed (see below) and then had Mg (II) added to a concentration of 7.5 mM. No other peaks were seen and the chemical shifts of the high field resonances were the same within ± 0.02 ppm. This correspondence makes it highly unlikely that tRNA which has undergone dialysis to remove Mg (II) is chemically altered from the original (e.g. by partial nuclease digestion).

For removal of Mg (II) $tRNA_1^{val}$ was dialyzed six times versus 100 mM EDTA, 15 mM phosphate, pH 7.0, three times versus 1 M NaCl, 15 mM phosphate, pH 7.0, three times versus 15 mM phosphate, pH 7.0 and (in the case of the thermal denaturation experiment) three times versus 200 mM NaCl, 5 mM phosphate, pH 7.0 or (for the NaCl addition experiment) three times versus 5 mM phosphate, pH 7.0. The samples were then lyophilized, dissolved in 100% D_2O, again lyophilized, redissolved in 0.4 ml of 100% D_2O and transferred to a 5 mm nmr tube.

Atomic absorption measurements yielded a value of 0.4 Mg ions per tRNA molecule for the nmr sample used in the thermal denaturation experiment and 0.6 Mg per tRNA in the salt addition sample.

Spectra for the thermal denaturation experiment were obtained using a Varian HR 220 with Nicolet 80 Fourier transform accessory. Temperatures were measured before and after each run using the chemical shift difference between the methylene and hydroxyl protons of ethylene glycol.[8] Other data

were obtained on the Stanford Magnetic Resonance Laboratory Bruker 360 MHz instrument operating in the Fourier transform mode. Chemical shifts were measured with respect to internal dioxane (1.4 mM) and are referenced to DSS (2,2-dimethyl-2-silapentane-5-sulfonate).

RESULTS AND DISCUSSION

A. Peak Assignments

The primary sequence of *E. coli* $tRNA_1^{val}$ is shown in Fig. 1. Modified bases which potentially give rise to resonances in the 4 to 0 ppm upfield "window" are hU 17, m^6A 37, m^7G 46, rT 54, and, possibly, 5-oxyacetic acid U 34.

1) High Temperature. 220 MHz Fourier transform nmr spectra of the upfield region (4 to 0 ppm from DSS) of $tRNA_1^{val}$ are shown in Fig. 2. Assignments are most straightforward for high temperature where the molecule is likely to be completely unfolded so that the modified base protons experience minimal effects from ring current anisotropies of adjacent nucleotides. Chemical shifts should be close to the values observed for the monomers.[4] Also line widths are narrow due to rapid segmental motion of the macromolecule. At the highest temperature observed, 85° C, five resonances, in addition to the dioxane reference, are found.

The farthest upfield peak is a narrow singlet at 1.73 ppm and is assigned to the methyl group of ribothymidine. This compares to 1.88 ppm for the methyl group of 2-deoxythymidine at 27° C. The triplet (J = 7.5 Hz) at 2.70 arises from the C5 methylene of hU since these protons produce a pseudo-

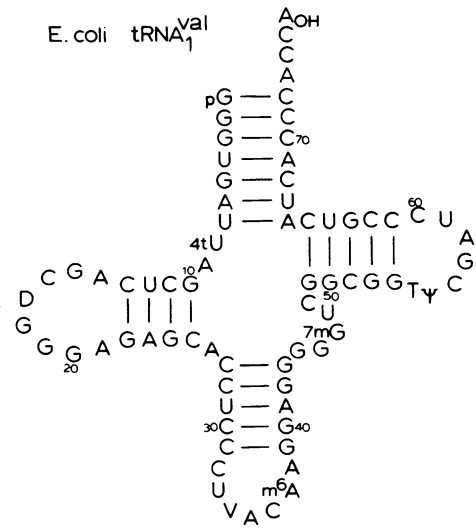

Fig. 1 Nucleotide Sequence of $tRNA_1^{val}$.

Modified nucleosides include: D, dihydrouridine; V, 5-oxyacetic acid U; m^6A, 6-methyl adenosine; m^7G, 7-methyl guanosine; T, ribothymidine; Ψ, pseudouridine; 4tU, 4-thiouridine.

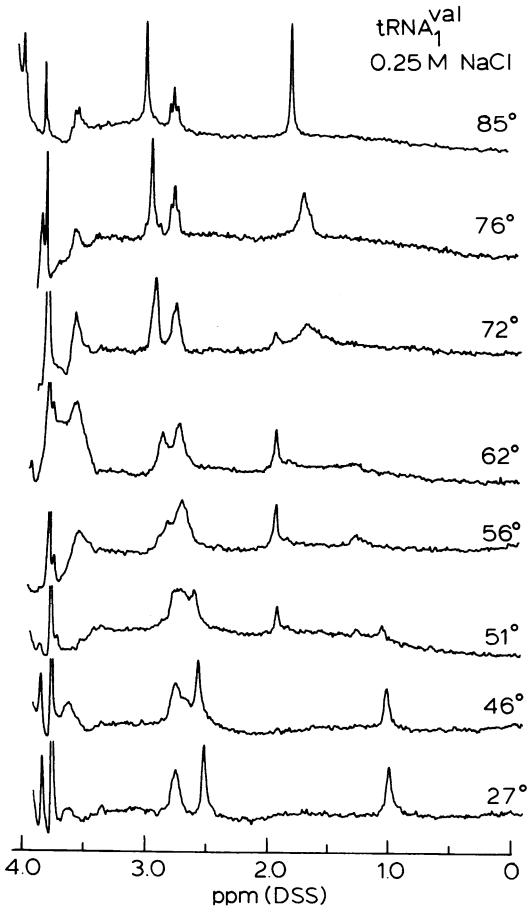

Fig. 2 Temperature Dependence of Upfield NMR Spectra.

Solutions contained 370 A_{260} tRNA/0.4 ml, 0.25 M NaCl, 10 mM P_i, pH 7.0, 0.4 Mg (II)/tRNA. Data were obtained with a Varian HR-220 spectrometer interfaced to a Nicolet NMR-80 Fourier transform accessory. 522 accumulations were averaged for each spectrum. The baseline in the region 3-4 ppm was adjusted to partially eliminate the ribose proton shoulder. For the sake of clarity spectra taken at 37°, 68° and 80° are not shown. Parameters for these spectra were 5.2 sec repetition time, 90° pulse, single phase detection, 4096 points in the frequency spectrum, 1 Hz line broadening imposed on the free induction decay.

triplet (J = 7.5 Hz) at 2.75 ppm in the nucleoside hU at 82° C. The sharp singlet at 2.92 ppm is assigned to the m^6A methyl protons; the methyl peak occurs at 3.09 ppm for the monomer at 82° C. A multiplet at 3.49 is assigned to the C6 methylene protons of hU since these occur at 3.55 ppm in the spectrum of hU at 82° C. The final peak in the upfield window is a sharp singlet at 3.92 ppm at 85° C and is assigned to the methyl group of m^7G; this peak is found at 4.11 ppm in the monomer at 82° C. The ratios of the areas of the m^6A methyl, hU C5 methylene, and rT methyl peaks are 3:1.8:3 at 85° C, in very good agreement with the expected ratio of 3:2:3. The m^7G methyl area is difficult to measure because the peak occurs on the steep slope of the ribose proton envelope.

2) Low Temperature Spectra. By following resonances from high temperature to low one can make assignments throughout the range.[4] This procedure

is not without pitfalls since in the present case slow exchange effects and multiple conformational intermediates serve to split resonances into several peaks during the transition. Nonetheless assignments at 27° can be made as follows: 1.0 ppm, ribothymidine methyl; 2.5, methyl of 6-methyl adenosine; 2.75, dihydrouridine C5 methylene; 3.65, hU C6 methylene; 3.82, 7-methyl guanosine methyl. The large singlet at 3.74 ppm is from the p-dioxane internal standard.

There is a possible ambiguity in the assignment of the m^6A methyl and hU C5 methylene since these peaks overlap and cross as the temperature is lowered from 85° to 27°. But the linewidth of the peak at 2.5 ppm is too narrow to accommodate a triplet of J = 7.5 Hz while that at 2.75 is well fit by such a multiplet. Our assignment of the m^7G methyl is in agreement with that reported by Daniel and Cohn in spectra of $tRNA_f^{met}$ (coli).[3] While the methylene resonance of the V base (5-oxyacetic acid U) in the "wobble" position is potentially in the neighborhood of 4.1 ppm[9] we find no evidence of it in low temperature spectra of $tRNA_1^{val}$.

3) Solution Conformation. Spectra of $tRNA_1^{val}$ at low temperature and 0.25 M NaCl support for the most part a solution structure similar to the crystal structure of $tRNA^{phe}$ from yeast.[10] A 0.8 - 0.9 ppm upfield shift of rT 54 is expected since the methyl is found in the crystal to be stacked over G 53 near the center of maximum shielding. m^6A 37 is shifted 0.5 ppm upfield in $tRNA_1^{val}$ at low temperature due mainly to shielding by A 38. Yeast $tRNA^{phe}$ has the Y base at position 37, consequently a direct comparison cannot be made between the two tRNAs. If one assumes, however, that m^6A has the same orientation with respect to A 38 that the Y base has, then the observed 0.5 ppm upfield shift is consistent in both sign and approximate magnitude with the shift expected from the crystal structure. Small downfield shifts (0.05 ppm for hU C5 methylene and 0.1 ppm for the C6) are observed for hU in the native conformation. These are quite reasonable considering the position of the hU in the crystal structure, i.e. unstacked, protruding away from adjacent bases in the dihydrouridine loop.

m^7G is found in the native structure at 3.82 ppm, approximately 0.2 - 0.3 ppm upfield of the denatured position. Kan et al.[4] calculated a net upfield shift of 0.21 ppm expected for m^7G methyl in $tRNA^{phe}$ (yeast) due to ring-currents of neighboring bases in agreement with the observed shift in $tRNA_1^{val}$ (coli).

These results suggest that in 4 key regions of the macromolecule the solution structure of $tRNA_1^{val}$ (coli) at low temperature (27° - 37°) and

moderate salt concentration (0.25 M NaCl) is closely similar to the crystal structure of $tRNA^{phe}$ (yeast). This conclusion must be tempered by the fact that present ring-current shift theory is not complete, that the x-ray crystal structure of $tRNA^{phe}$ (yeast) is not completely refined and that, even though their sequences are largely similar, any extrapolation of the $tRNA^{phe}$ structure to $tRNA_1^{val}$ has pitfalls.

B. Thermal Denaturation

These experiments were done on a sample of $tRNA_1^{val}$ which had been extensively dialyzed against EDTA and high salt concentrations to remove Mg^{2+} (see Materials and Methods). The final nmr sample contained 790 A_{260} units/ml tRNA, 250 mM NaCl, 19 mM P_i, pH 7. Atomic absorption analysis showed 0.4 Mg^{2+} ions per tRNA molecule.

As the temperature is raised the macromolecule unfolds, producing new magnetic environments for the modified bases. The behavior of each resonance provides a detailed measure of the thermodynamics (and in some cases, kinetics) of conformational changes in the vicinity of that particular nucleotide. A glance at the spectra of Fig. 2 makes it clear that the thermal unfolding of $tRNA_1^{val}$ is not a simple helix-to-coil process.

1) Ribothymidine Methyl. The rT methyl group presents the most interesting and easily followed behavior of the resonances. A single peak arises from these protons (at 1.0 ppm) as the temperature is increased from 27° to 46° C, at which point a small singlet is evident at 1.9 as well. As the temperature is raised further to 51° C two additional peaks appear, one at 1.8 and the other at 1.25 ppm. An increase in area occurs in the 1.9 ppm peak with a compensating decrease in area for the 1.0 ppm peak. As the temperature is raised to 62.5° C the peak at 1 ppm has disappeared, the 1.9 ppm resonance has reached its maximum amplitude and is beginning to decrease in area, while peaks at 1.8 and 1.25 ppm are broadening significantly and are increasing in area, the latter especially. At 68° C the area of the 1.9 ppm peak has decreased markedly, and the 1.8 and 1.25 ppm peaks have coalesced into a broad resonance. Above 72° C only one peak is evident which shifts downfield with further temperature increases.

It appears from this behavior that four distinct sites can be assigned to the rT methyl group. The 1.0 ppm site corresponds to a "native" conformation with tertiary structure intact. Spectra of $tRNA_1^{val}$ from this and other samples have shown that the 1.0 ppm site is promoted by addition of Mg^{2+} and by low temperature. The 1.8 ppm site which exists at high temperatures represents the "random coil" conformation with loss of tertiary and

secondary structure. The peaks at 1.25 and 1.9 ppm correspond to intermediate structures. 1.9 ppm is the same shift as free ribothymidine indicating that rT is no longer stacked on G 53 and that the A 58 - rT 54 tertiary base pair probably is lost.

Relative populations among the four sites were determined from the peak areas, or chemical shifts, depending on whether slow or fast exchange pertained. A plot of relative populations versus temperature for the rT methyl is shown in Fig. 3. Two equilibria can be followed easily from the populations. The first is exchange between the native conformation and others ($1.0 \rightleftarrows 1.25 + 1.8 + 1.9$). This transition has a T_m of $51 \pm 5^{\circ}$ and an enthalpy change $\Delta H = 125 \pm 15$ kcal/mole. The second is between random coil and the other, folded forms ($1.8 \rightleftarrows 1.0 + 1.25 + 1.9$) with $T_m = 70 \pm 5^{\circ}$ and $\Delta H = 70 \pm 15$ kcal/mole.

The value of ΔH for the 51° transition (125 kcal/mole) is certainly larger than what one would predict from the loss of tertiary structure alone or from the loss of any one stem. Apparently the salt concentration used for this set of experiments was sufficient to stabilize tertiary structure above the inherent T_m of some of the stem regions so that these regions and the tertiary structure melted simultaneously. From its T_m of 70^{0} and ΔH of 70 kcal/mole the final rT transition appears to include the helix-to-coil shift of the TΨC stem.

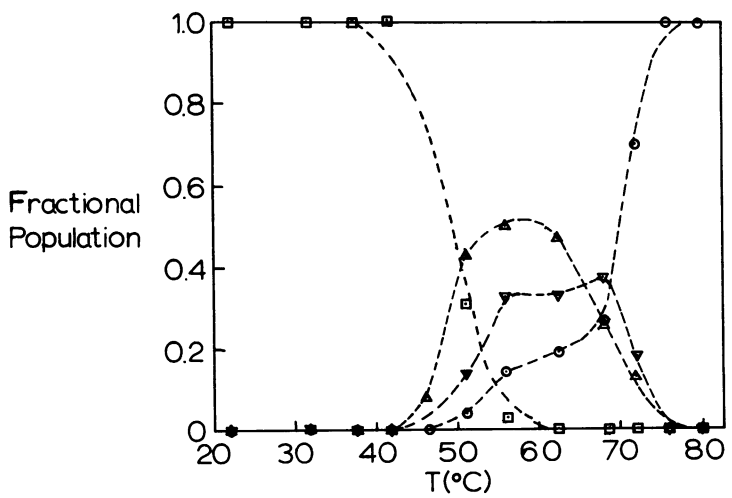

Fig. 3 Populations vs. Temperature for rT Sites.

Relative populations for each of the four sites of the rT methyl group: 1.0 ppm, 1.25 ppm, 1.9 ppm, 0 1.8 ppm

We have observed multiple conformations in thermal denaturation nmr spectra of $tRNA_1^{val}$ run under a variety of solutions conditions (ref. 2 and R. V. Kastrup (1976) Ph.D. Thesis, University of Illinois). In general approximately the same chemical shift values are found for the intermediate structures but their proportions vary with salt and Mg(II) concentration. For example, a Mg(II) concentration of 6 mM raises the T_m values of most transitions compared to those reported here for low Mg(II) concentration. However, the same number and types of sites are seen for each spin system of the modified bases.

In an earlier study of $tRNA_1^{val}$ thermal denaturation[2], we used conditions of minimum salt concentration in an effort to spread out melting of the molecule over a wider temperature range. The sample there was dialyzed into a dilute P_i buffer and then concentrated so that the Na^+ ion concentration was only .045 M more than needed for counterions. In this present work the sample was prepared at the nmr concentration then dialyzed against a 0.20 M NaCl buffer to yield an nmr sample having approximately 5 times the free Na^+ concentration used in our previous study. Multiple intermediates were also observed in the early spectra. However, because of the complexity of the rT thermal transition, 2 ribothymidine peaks were not recognized as arising from the tRNA. Furthermore under the conditions used, at 38° the 1.25 ppm rT peak contained the most area, as opposed to our present results where the 1.0 ppm peak dominates at that temperature.

Kan et al.[4] have recently made a detailed proton nmr study of the thermal denaturation of $tRNA^{phe}$ (yeast). When the sample is 10 mM in Mg(II) they find that as the molecule is cooled from high temperature the rT methyl first broadens then splits into two resonances. At 50°, where the structure is apparently native, these peaks are at 1.0 and 1.5 ppm and contain, respectively, 60% and 40% of the rT area. This behavior is similar in some respects to that seen for $tRNA_1^{val}$, except that the rT group of $tRNA^{phe}$ does not sample as many different intermediate conformations, and the rT region is partitioned between 2 different conformations in the native structure rather than being predominantly in one as with $tRNA_1^{val}$. Interestingly, $tRNA^{phe}$ (coli) (to be published) shows the rT resonance split into 2 peaks at low temperature.

2) m^6A Methyl Group. Transitions involving the m^6A methyl and hU C5 methylene resonances are more difficult to follow since these resonances overlap at intermediate temperature. At the lowest temperature (27° C) the m^6A methyl group occurs primarily as a singlet at 2.5 ppm, with a small fraction farther downfield. As the temperature is increased to 46° C the 2.50 ppm singlet moves downfield to 2.55 ppm. Since the m^6A is in a single

stranded loop region the downfield shift almost certainly represents partial unstacking of the base as the temperature is raised. Single strand unstacking occurs as an extremely rapid hypochromicity change in temperature jump experiments[11] with rate constants on the order of 10^6 sec^{-1} or greater, accounting for the fast exchange behavior seen in the nmr spectra (shifting of the peak without broadening or loss of amplitude). At 46° C there is an increase in area around 2.65 ppm and loss at 2.55. By 56° C the narrow 2.55 ppm peak has disappeared and most of the m^6A methyl population has moved to 2.65 - 2.7 ppm. Between 56° and 85° the peak shifts progressively downfield to 2.92 ppm. Two possibilities account for the behavior of m^6A at temperatures higher than 56°. Either there is a slow exchange conformational equilibrium between 2.65 and 2.85 ppm up to 76° followed by single strand unstacking, or, alternatively, the methyl peak moves downfield from 2.65 ppm due solely to single strand unstacking. The overlap of the hU C5 methylene and m^6A methyl peaks prevent a clear distinction between these possibilities. In either case the anticodon helix is disrupted during the denaturation process.

3) hU C5 and C6 Methylenes. During thermal denaturation only two peak positions are discernable for the hU C5 methylene group. An unresolved multiplet at 2.74 to 2.75 ppm is present in the spectra until 51° C, at which point the peak, or peaks in the region from 2.6 to 2.8 ppm become very diffuse. At 62.5° C the situation is clearer and the hU C5 methylene resonance is seen at 2.70. A triplet with 7.5 Hz coupling constant remains in this region through 85° C. This C5 transition coincides with an upfield shift of the hU C6 methylene multiplet from 3.65 ppm to 3.49 ppm between 46° C and 56° C.

The dihydrouridine peaks are unusual in that, unlike most nucleic acid resonances, they shift upfield (more net shielding) as the molecule unfolds. The hU transition midpoint (for both C5 and C6 protons) is 51 ± 5° C. There are not enough data points, however, to determine accurately a ΔH value.

4) m^7G Methyl Group. At 27° the m^7G methyl group gives rise to a sharp singlet resonance at 3.82 ppm. At 51° two peaks are found, one at 3.82 and the other at 3.72 ppm. At 56° and 62.5° C there is one predominant resonance, occurring at 3.72 ppm. From 68° to 85° the m^7G methyl group moves downfield from 3.72 to 3.9 ppm in a fast exchange equilibrium. Three magnetic environments are observed for m^7G corresponding to the native structure at 3.82 ppm, an intermediate site at 3.72 ppm and a fully denatured form at 3.9 ppm and even farther downfield at higher temperature. The peak from the m^7G methyl occurs on the shoulder of the large 5' ribose proton envelope and very close

to the dioxane reference. Consequently, accurate area measurements, and hence population estimates for the various sites, cannot be made. It is noteworthy that the midpoint of the slow exchange transition occurs at the same temperature (~ 51° C) as the hU methylene proton's transition and the low termperature transition of the rT methyl group. The fast exchange downfield shift does not occur to an appreciable extent until above 68° C, i.e., the same general temperature region as the high temperature transition of the rT methyl group.

5) Exchange Lifetimes. Effects of chemical exchange on nmr lineshape are clearly seen for the ribothymidine group. From 46° to 72° rT undergoes slow exchange (individual, non-averaged peaks) between 1.9 ppm and sites with shifts upfield. The line width of the 1.9 ppm peak can be used to estimate an exchange lifetime in this site since broadening over and above the intrinsic dipolar width and instrumental contribution is due to the exchange process.[12] Using the line width of dioxane as an inhomogeneity reference and the width of the 85° rT methyl peak as a minimum dipolar contribution the average lifetime in the 1.9 ppm site is calculated to be $\geq$ 140 msec between 51° and 62°.

6) Thermal Hysteresis. Thermally unfolded $tRNA_1^{val}$ does not necessarily return completely to its native conformation when cooled. As shown in Fig. 4a slow cooling (sample left at room temperature) following heating at 85° C yielded a spectrum in which all of the native and intermediate sites were at least partially populated. Addition of 10 mM $MgCl_2$ and rapid cooling (sample placed in an ice bath) following heating at 64° C showed greatly reduced intensity in most of the intermediate sites (Fig. 4b) although the spectrum did not correspond exactly to the spectrum obtained before melting, (4c). The 1.9 ppm site population in particular diminished in the spectrum of the rapidly cooled material. Differences in the region 6.5 to 9 ppm are due mainly to exchange of purine H-8 protons for deuterium at high temperature.

The differences between the spectra taken after rapid and slow cooling point out the fact that intermediate conformations, although they are not the most stable thermodynamically, may be extremely slow in returning to the native conformation because of high activation energy barriers. Electrostatically renatured material (NaCl added to low salt concentration $tRNA_1^{val}$ solutions) also showed some evidence of multiple renaturation paths (see below).

C. Effects of Salt Concentration

Figure 5 shows nmr spectra from the 4 to 0 ppm region of E. coli

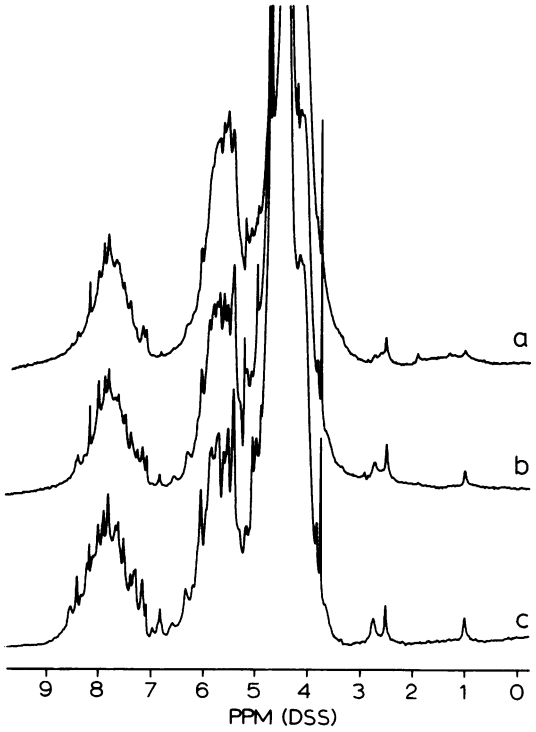

Fig. 4 NMR Spectra Showing Thermal Hysteresis.

Conditions are those of Fig. 2. All 3 spectra were taken at 27° C. Spectrum c was obtained before the sample had been heated. a was recorded after the sample had been heated at 85° for 1 hr then cooled at room temperature for 1 hr. Spectrum b was taken subsequent to a. 10 mM $MgCl_2$ was added to the sample from a, and the solution was heated at 64° for 100 min, cooled rapidly in ice and then reincubated at 37° for 3.5 hr, reheated at 65° for 45 min, cooled in ice and incubated at 37° for 5.5 hr. Spectrum b was then recorded at 27° C.

$tRNA_1^{val}$ at various Na^+ and Mg^{2+} concentrations. Spectrum a, taken at the lowest salt concentration (20 mM free Na^+, 110 mM Na^+ "bound" as counter ions, 0.6 Mg^{2+} per tRNA), clearly shows the presence of non-native conformations. The hU C5 methylene peak appears at 2.70 rather than at the 2.75 ppm native structure position. The m^6A methyl group is seen at 2.60 rather than the native site of 2.5 ppm. Using the sum of the areas of the peaks at 2.70 and 2.60 ppm as 5 protons, the total area of the sharp peaks at 1.8, 1.2 and 1.0 ppm, plus the broad peaks at 1.2 and 0.85 ppm corresponds to 3.0 ± 0.3 protons. It is most reasonable to conclude that the 5 peaks arise from different environments of the rT base. Since separate peaks are observed for the rT conformations present in solution (slow exchange on the nmr time scale) the lifetime in each site can be assigned a lower limit as > 50 msec. It is interesting that the 1.9 ppm rT site is unpopulated in these low salt concentrations. This suggests that the 1.9 ppm resonance does not arise from extended forms observed by Cole et al. in low salt concentrations for several tRNA's.[13]

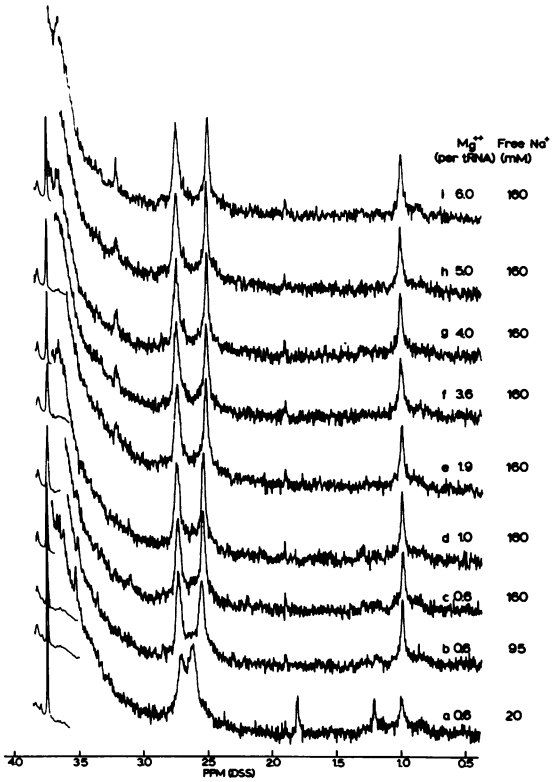

Fig. 5 Effects of Salt and Divalent Metal Ion Concentration.

Initial concentrations in the nmr tube were 250 A_{260} tRNA/0.4 ml, 20 mM free Na^+, 19 mM P_i, pH 7.0. Spectra were obtained in the Fourier transform mode using the Stanford Magnetic Resonance Laboratory 360 MHz spectrometer. All spectra were run at 27° C. Stepwise additions of NaCl and $MgCl_2$ were made using stock solutions of the salts in D_2O. Total volume added did not exceed 50 µl. Spectral parameters were 5.8 sec between pulses, 90° pulse, single phase detection, 8192 points in the frequency spectrum, 0.5 Hz broadening. 256 scans.

Addition of 75 mM NaCl to the solution produced substantial changes in the $tRNA_1^{val}$ conformation, as shown by spectrum b. The hU C5 and C6 methylenes, m^6A methyl and rT methyl groups have returned predominantly to their native sites, although the rT methyl does show a small peak at 1.9 ppm and some area in the 1.2 and 0.85 ppm regions. The m^7G methyl still shows two peaks, although in substantially different ratio than the lower salt concentration spectrum. Further addition of NaCl (to 160 mM free Na^+) and addition of Mg^{2+} (to 6 Mg^{2+} per tRNA) causes the m^7G methyl to shift completely to the 3.83 ppm site and enhances the formation of native structure in the other sites.

ACKNOWLEDGEMENTS

We thank Ms. M. Beth Lepinski for excellent technical assistance. The 360 MHz spectra were obtained at the Stanford Magnetic Resonance Laboratory with the help of Drs. S. L. Patt and W. W. Connover. The Stanford facility is supported by NSF grant GR 23633 and NIH grant RR 00711. Dr. Prem Sattsangi kindly provided a sample of 6-methyladenosine. This work was supported by NIH grant GM 18038 to PGS.

REFERENCES

+ Present address: Department of Chemistry, University of Rochester, Rochester, New York 14627.
* Present address: Oklahoma Medical Research Foundation, 825 N. E. 13th St., Oklahoma City, Oklahoma 73104.

1. Kearns, D. R., and Shulman, R. G. (1974) Acc. Chem. Res. 7, 33-39
2. Kastrup, R. V., and Schmidt, P. G. (1975) Biochemistry 14, 3612-3618
3. Daniel, W. E., and Cohn, M. (1976) Biochemistry 15, 3917-3924
4. Kan, L. S., Ts'O, P. O. P., Sprinzl, M., v. d. Haar, F., and Cramer, F. (1977) Biochemistry 16, 3143-3154
5. Roy, K. L., Bloom, A., and Söll, D. (1971) Proc. in Nucleic Acid Res. 2, 524-541
6. Nishimura, S. (1971) Proc. in Nucleic Acid Res. 2, 542-564
7. Holmes, W. M., Hurd, R. E., Reid, B. R., Rimerman, R. A., and Hatfield, G. A. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 1068-1071
8. Van Geet, A. L. (1968) Anal. Chem. 40, 2227-2229
9. Murao, K., Saneyoshi, M., Harada, F., and Nishimura, S. (1970) Biochem. Biophys. Res. Commun. 38, 657-662
10. Quigley, G. J., and Rich, A. (1976) Science 194, 796-806
11. Porschke, D., and Eigen, M. (1971) J. Mol. Biol. 62, 361-381
12. Gutowsky, H. S., and Holm, C. H. (1956) J. Chem. Phys. 25, 1228-1234
13. Cole, P. E., Yang, S. K., and Crothers, D. M. (1972) Biochemistry 11, 4358-4368