RNA structure analysis using T2 ribonuclease: detection of pH and metal ion induced conformational changes in yeast tRNA^{Phe}

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Received 14 May 1984; Revised and Accepted 13 August 1984

ABSTRACT

We describe the use of an enzymic probe of RNA structure, T2 ribonuclease, to detect alterations of RNA conformation induced by changes in Mg^{2+} ion concentration and pH. T2 RNase is shown to possess single-strand specificity similar to S1 nuclease. In contrast to S1 nuclease, T2 RNase does not require divalent cations for activity. We have used this enzyme to investigate the role of Mg^{2+} ions in the stabilization of RNA conformation. We find that, at neutral pH, drastic reduction of the available divalent metal ions results in a decrease in the ability of T2 RNase to cleave the anticodon loop of tRNA^{Phe}. This change accompanies an increase in the cleavage of the molecule in the T_ψC and in the dihydrouracil loops. Similar treatment of *Tetrahymena thermophila* 5S ribosomal RNA shows that changes in magnesium ion concentration does not have a pronounced effect on the cleavage pattern produced by T2 RNase. T2 RNase activity has a broader pH range than S1 nuclease and can be used to study pH induced conformational shifts in RNA structure. We find that upon lowering the pH from 7.0 to 4.5, nucleotide D16 in the dihydrouracil loop of tRNA^{Phe} becomes highly sensitive to T2 RNase hydrolysis. This change accompanies a decrease in the relative sensitivity of the anticodon loop to the enzyme. The role of metal ion and proton concentrations in maintenance of the functional conformation of tRNA^{Phe} is discussed.

INTRODUCTION

An important approach to the study of the structure, in solution, of RNA molecules involves the use of enzymic and chemical probes of RNA structure(1). Studies using enzymic probes can generate detailed structural information on large RNA molecules, which cannot be obtained using optical, magnetic resonance or crystallographic techniques(2). Enzymes of high structural specificity, such as single-strand specific S1 nuclease and the double-strand specific cobra venom RNase(CVR), are used to digest end-labeled RNA molecules under conditions of limited cleavage to determine which nucleotides are involved in single-strand or double-helical regions, respectively(2,3). The results obtained using structure probes of complementary specificity are used, together with thermodynamic considerations, to formulate RNA secondary structure models. Such an approach has been valuable in investigating the structure of various RNAs, including the

rabbit globin mRNAs(3,4) and the 5S ribosomal RNAs from Dictyostelium discoideum and Bombyx mori(5).

Central to the development of the RNA structure mapping approach has been the use of yeast tRNA^{Phe} as a model compound. The degree of precision to which the structure of yeast tRNA^{Phe} is known, primarily from x-ray crystallographic analysis, establishes it as the most thoroughly characterized naturally occurring RNA molecule available for study(6).

Enzymic probes have not been used as yet to provide information on the conformational behavior of large RNA molecules due, in part, to the strict pH and divalent cation requirements of enzymes such as S1 nuclease and CVR(7,8). Thus, enzymic probes have been of little use in describing conformational changes in RNA molecules resulting from changes in ionic strength, ionic composition, pH and temperature.

Physical studies by numerous investigators have documented considerable conformational flexibility in natural RNA molecules such as the tRNAs. Jones and Kearns(9) used proton NMR chemical shifts to study conformational changes in tRNA^{Phe} that accompany changes in pH. Crothers and co-workers(10,11) have documented a dependence of the thermal stability of tRNA molecules on pH and ionic strength. Small ligands such as divalent metal ions, and polycations such as spermidine are important in the stabilization of RNA structure, as shown by x-ray analysis for tRNA^{Phe}(12). X-ray crystallographic data have been interpreted to support a model having at least four tightly bound Mg²⁺ atoms in the crystal structure of yeast tRNA^{Phe}. These studies are consistent with the notion that metal ions function to stabilize tertiary interactions in tRNA^{Phe}.

T2 RNase, a well studied endoribonuclease(13,14), does not require a divalent cation cofactor. We present data obtained using T2 RNase indicating that it is an efficient and accurate single-strand specific probe of RNA structure. In addition, these studies provide evidence that Mg²⁺ ions are important in the determination of the function of the anticodon loop.

MATERIALS AND METHODS

Yeast tRNA phenylalanine was purchased from Boehringer Mannheim. Tetrahymena thermophila 5S ribosomal RNA was prepared as described by Kumazaki et al.(15). Ribonuclease T2(Grade VI) was purchased from Sigma Chemical Co. All other enzymes and reagents were obtained and used as described previously(1) unless specifically indicated below.

Cobra venom ribonuclease digestions were performed in 5 μ l reaction volumes containing 1.25 μ g total RNA and 20mM tris•HCl(pH 7.0), 100mM sodium chloride

and 2mM MgCl₂. T2 RNase digestions in the presence of Mg²⁺ were conducted in buffer identical to that used for CVR(16) except where specifically noted. For comparison of structure reactions following chelation of divalent cations, parallel incubations were prepared, as follows: aliquots of end-labeled tRNA^{Phe}(20 kcpm Cerenkov) were dissolved in 6μl of distilled deionized water and heated to 90°C. Following a 1 minute incubation at this temperature 2μl of a solution of 10mM Na₂EDTA of 10mM MgCl₂ were added to the RNA solution. After an additional minute at 90°C, 2μl of 5x buffer containing 100mM tris·HCl(pH 7.0), 500mM sodium chloride and 125μg of carrier tRNA were added and the samples allowed to equilibrate slowly to a final temperature of 37°C. Following the equilibration period, enzyme was added. Aliquots were taken as a function of time as described in the figure legends. Reactions were quenched by the addition of an equal volume of a solution containing 9M Urea, 0.1% bromphenol blue, 0.1% xylene cyanol, 10μg per μl carrier tRNAs and 40mM Na₂EDTA. Reactions conducted at low pH were prepared as described above except that the 5x buffer contained 20mM sodium acetate(pH 4.5) instead of tris·HCl. Reactions involving ferric ion were conducted at pH 4.5 using the same thermal denaturation protocol described above except that 2μl of a solution of 7.9mM ferric chloride was used in place of magnesium chloride. These concentrations of Na⁺, Mg²⁺, and Fe³⁺ ions were adjusted so as to maintain conditions of constant ionic strength.

Gel electrophoresis of the digestion products was performed at 1000 volts constant voltage, at an initial current of approximately 20 mA. This protocol minimizes band distortion and is optimal for quantitative scanning microdensitometry(17). Autoradiograms were exposed for times sufficient to produce a maximum band optical density less than or equal to one O.D. measured at 500 nanometers. Autoradiograms were scanned using a Jarrell Ash model 23-100 densitometer with slit dimensions of 2nm x 15μm at a wavelength of 500nm. Output from the densitometer was collected through an interface to a NEC-APL microcomputer and the output, initially expressed as % transmittance, was converted to absorbance. Peak densities for all scans were reproducible to ±5%. Scanning microdensitometric analysis of autoradiograms was performed under specific calibration conditions determined to provide a linear response for concentration versus film optical density. The details of the calibration procedures as well as the algorithms used for data acquisition, band deconvolution, area measurement and curve smoothing will be published elsewhere(17).

Figure 1. Mg^{2+} ion dependence of the sensitivity of $tRNA^{Phe}$ towards T2 ribonuclease. Samples of 5'-end labeled $tRNA^{Phe}$ (20kcpm Cerenkov) were digested with structure and sequence specific endonucleases under conditions detailed in Methods. Following digestion, reactions were quenched as described and electrophoresed on a 20% denaturing polyacrylamide gel (35cm x 40cm x 0.33cm). Lanes 1, 2, T1 RNase, 5×10^{-4} units per μg of RNA at $60^\circ C$ for 1 and 3 minutes respectively; Lane, 3, alkaline hydrolysis in the presence of 50mM $NaHCO_3/Na_2CO_3$ buffer, pH 9.0 for 10 minutes at $90^\circ C$; lane 4, S1 nuclease, 0.1 units per μg RNA, 3 minutes at $37^\circ C$ in the presence of 2mM $ZnCl_2$ ions; lane 5, cobra venom ribonuclease, 8×10^{-4} units per μg RNA for 5 minutes at $37^\circ C$ in the presence of 2mM Mg^{2+} ions; lanes 6-8, T2 RNase, 1.5×10^{-4} units per μg RNA, $37^\circ C$ and in the presence of 2mM Mg^{2+} ions for 1, 3 and 8 minutes respectively; lanes 9-11, T2 RNase, 1.5×10^{-4} units per μg RNA for $37^\circ C$ and in the presence of 2mM Na_2EDTA for 1, 3 and 8 minutes respectively (see Methods for details). The positions of guanylate residues are indicated by the numbers along the left side of the figure.

RESULTS

Figure 1 shows results obtained following digestion of 5'-end-labeled tRNA^{Phe} with the structure specific endonucleases S1, CVR and T2 RNase. Comparison of the S1 nuclease digestion products in lane 4 with the T2 RNase products in lanes 6-8 shows that both enzymes selectively hydrolyze the anticodon loop. The T2 RNase bands have slightly greater electrophoretic mobility than the S1 nuclease and CVR bands since they have a 3' terminal phosphate(14). S1 nuclease and T2 RNase have slightly different reactivity to bases in the anticodon region of tRNA^{Phe}. S1 nuclease favors cleavage of nucleotide A36 compared with T2 RNase which, in addition to the common band at position A35, favors cleavage of nucleotide U33. Neither T2 RNase or S1 nuclease cleaves phosphodiester bonds sensitive to double-strand specific CVR in tRNA^{Phe}. A comparison of the T2 RNase reactions, following reduction of the available Mg²⁺ in the reaction mixture by chelation with EDTA, is shown in lanes

Figure 2. Densitometric analysis of the effect of Mg²⁺ on the distribution and frequency of the T2 RNase cleavage products of 5'-end labeled tRNA^{Phe}. Lanes 8 and 11 of the gel autoradiogram shown in figure 1 were analyzed by scanning microdensitometry. Panel A, 2mM Mg²⁺ pH 7.0; panel B, 2mM Na₂EDTA, pH 7.0.

9-11. Chelation of Mg^{2+} with EDTA results in an apparent decrease in the ratio of cleavages occurring at the anticodon loop relative to cleavages occurring in the $T\psi C$ loop. Quantitation of digestion products produced by T2 RNase in the presence (panel A) and following chelation of Mg^{2+} (panel B) is shown in figure 2. Densitometric analysis shows that T2 RNase cleavage in the anticodon loop is favored by the presence of Mg^{2+} .

Figure 3 illustrates the T2 RNase sensitivity of 5' end-labeled $tRNA^{Phe}$ with respect to metal ions and pH. Lanes 3-5 and 6-8 represent $+Mg^{2+}$ and chelated $Mg^{2+}(+EDTA)$ reactions, respectively, run identically to those shown in figure 1. Lanes 9-11 demonstrate partial restoration of anticodon loop

Figure 3. pH and metal ion dependence of the sensitivity of $tRNA^{Phe}$ to T2 RNase. Samples of 5'-end labeled $tRNA^{Phe}$ (20kcpm Cerenkov) were digested with T2 RNase under conditions described in Methods. Digestion products were electrophoresed as described in the legend to figure 1. Lane 1, T1 ribonuclease; lane 2, alkaline hydrolysis; lanes 3-5, T2 RNase, 2mM Mg^{2+} , pH 7.0 for 1, 3 and 5 minutes respectively; lanes 6-8, T2 RNase, 2mM Na_2EDTA , pH 7.0, for 1, 3 and 5 minutes respectively; lanes 9-11, T2 RNase, 2×10^{-4} units per μg RNA, 1mM $FeCl_3$ at pH 7.0 for 2, 5 and 8 minutes respectively; lane 12, S1 nuclease; lane 13, minus enzyme, 2mM Mg^{2+} , pH 7.0; lane 14, minus enzyme + 2mM Na_2EDTA , pH 7.0; lane 15, minus enzyme, 2mM Mg^{2+} , pH 4.5 for 1, 3 and 5 minutes respectively; lanes 16-18, T2 RNase, 4×10^{-5} units per μg RNA, 2mM Mg^{2+} , pH 4.5 for 1, 3 and 5 minutes respectively. The positions of guanylate residues are indicated along the left side of the figure.

sensitivity to T2 RNase by FeCl_3 . FeCl_3 does not change the T2 RNase sensitivity of dihydrouracil and T ψ C loop residues.

Differences in T2 RNase sensitivity of tRNA^{Phe} resulting from a pH change from 7.0 to 4.5 in the presence of Mg^{2+} are shown in lanes 16–18 of figure 3. A significant enhancement of the sensitivity of the dihydrouracil residue D16 towards T2 RNase is observed at the lower pH. Lanes 13–15 show that no fragmentation of the RNA has occurred prior to enzyme addition. The pH induced change of sensitivity of tRNA^{Phe} to T2 RNase was quantitated by scanning microdensitometry as shown in figure 4. Dihydrouracil residue D16 becomes highly sensitive to T2 RNase catalyzed hydrolysis at the pH 4.5. Other regions of the molecule, toward the 5' terminus, become slightly more sensitive to T2 RNase at low pH. Data confirming and extending the above results, using 3' end-labeled tRNA^{Phe} as substrate, is shown in figure 5. While many bands remain

Figure 4. Densitometric analysis of the effect of pH on the distribution and frequency of T2 RNase cleavage products of 5'-end labeled tRNA^{Phe}. Lanes 4 and 18 of the gel autoradiogram shown in figure 3 were quantitated by scanning microdensitometry as described in Methods. Panel A, lane 18, pH 4.5, 2mM Mg^{2+} ; Panel B, lane 4, pH 7.0, 2mM Mg^{2+} . Numbers refer to the positions of nucleotide residues counting from the 5' terminus.

relatively insensitive to pH variation from 7.0 to 4.5, it is clear that the relative sensitivities of the anticodon, DHU and T ψ C loops are altered by this pH shift. These data are summarized in figure 8.

5S ribosomal RNA from *Tetrahymena thermophila* was labeled at its 3' terminus and the sensitivities of single-stranded regions towards T2 RNase were

Figure 5. pH dependence of the sensitivity of 3'-end labeled tRNA^{Phe} to T2 RNase. Samples of 3'-[³²P]-labeled tRNA^{Phe} (20 kcpm Cerenkov) were digested with sequence and structure specific enzymes under the conditions described in the legend to figure 1 and in Methods. The products of the digestion reactions were resolved by polyacrylamide gel electrophoresis as detailed in figure 1. Lane 1, minus enzyme; lane 2, T1 RNase; lane 3, alkaline hydrolysis; lane 4, U2 RNase, 3×10^{-4} units per μ g RNA at 60°C for 10 minutes; lane 5, S1 nuclease; lane 6, cobra venom RNase; lanes 7-9, T2 RNase 4×10^{-5} units per μ g RNA, 2mM Mg²⁺, pH 4.5 for 1, 5 and 8 minutes respectively at 37°C; lanes 10-12, T2 RNase 1.5×10^{-4} units per μ g RNA, 2mM Mg²⁺ pH 7.0 for 1, 5 and 8 minutes respectively at 37°C. The numbers to the left of the figure refer to the positions of guanylate residu

Figure 6. Metal ion dependence of the sensitivity of 3'-end labeled *Tetrahymena* 5S ribosomal RNA to T2 ribonuclease. Samples of 3'-[32 P]-labeled 5S rRNA were subjected to digestion with sequence and structure specific endonucleases as described in the legend to figure 1 and in Methods. Following digestion, the reactions were quenched and the products of the digestion reactions resolved on a 15% polyacrylamide gel (30cm x 90cm x 0.27) run at 2500 v until the bromphenol blue marker dye had migrated 60 cm. Lane 1, minus enzyme; lane 2, T1 RNase; lane 3, alkaline hydrolysis; lane 4, U₂ RNase 3×10^{-4} units per μ g RNA, 10 minutes at 60°C; lanes 5-7, S1 nuclease, 5, 2 and 1 minute respectively; lanes 8-10; T2 RNase, 2mM Mg²⁺ pH 7.0, 1, 3 and 5 minutes respectively; lanes 11-13, T2 RNase, 2mM Na₂EDTA, pH 7.0 for 5, 3 and 1 minutes respectively. The numbers to the left of the figure refer to guanylate and adenylate residue positions.

measured in the presence of and following chelation of Mg^{2+} . Figure 6 shows the results of digestion of *T.thermophila* 5S rRNA with structure and sequence specific enzymes including S1 nuclease and T2 RNase with and without Mg^{2+} . As was the case with yeast tRNA^{Phe}, there is a very close correspondence between S1 nuclease (lanes 5-7) and T2 RNase (lanes 8-13) cleavage sites. Visual inspection of the lanes which correspond to equivalent extents of digestion in the presence of Mg^{2+} (lanes 8, 9, and 10) or following chelation of Mg^{2+} with

Figure 7. Densitometric analysis of the distribution of T2 RNase and S1 nuclease digestion products of *Tetrahymena thermophila* 5S ribosomal RNA. Lanes 5, 8 and 13 of the autoradiogram shown in figure 6 were quantitated by scanning microdensitometry. Panel A, lane 13, 2mM Na_2EDTA , pH 7.0; Panel B, lane 8, 2mM Mg^{2+} , pH 7.0; panel C, lane 5, S1 nuclease, 2mM Zn^{2+} , pH 4.5.

EDTA(lanes 11, 12, and 13) reveals slight shifts in sensitivity of 5S rRNA to the structure probe. These changes include increases in T2 RNase sensitivity of nucleotides 42-43, 101-102 and the 3' terminal nucleotides of the molecule. Scanning densitometric analysis of equivalently digested samples within the top two thirds of the autoradiogram are shown in figure 7 and reveal only slight Mg^{2+} dependent differences in digestion rate at the nucleotide positions mentioned above.

DISCUSSION

Experiments were undertaken to study the utility of T2 RNase as a probe of RNA structure. Unlike S1 nuclease and cobra venom RNase, T2 RNase does not require divalent cations for activity(13,14). In addition T2 has a broad pH range, from 4-7, and is stable over a wide temperature range, from 10 to 75°C. T2 RNase, therefore, has potentially favorable characteristics for use as an RNA conformation probe. We begin to exploit these properties in this work.

T2 RNase is preferable to S1 nuclease for pH dependent RNA conformation studies. T2 RNase and S1 nuclease both have pH optima of 4.5(7,13). However,

Figure 8. Summary of the sensitivity of tRNA^{Phe} to S1 nuclease, T2 RNase and CVR. The meanings of the symbols are as indicated in the figure.

T2 RNase retains considerable activity at pH 7.0. Equivalent extents of digestion by T2 RNase of end-labeled tRNA^{Phe} were obtained with only a 4 fold increase in enzyme concentration at pH 7.0 relative to the pH 4.5. Studies are currently in progress in our laboratory using T2 RNase to monitor the thermal unfolding of tRNA^{Phe}. Thermal denaturation studies using S1 nuclease are limited to temperatures below 50°C. Zn²⁺ ions, which are required for S1 nuclease activity, catalyze RNA hydrolysis at a significant rate at temperatures greater than about 50°C(18).

The structural specificity of T2 RNase is very similar but not identical to that of S1 nuclease. Data presented above on tRNA^{Phe} demonstrate that T2 RNase cleaves D16 at pH 4.5, whereas S1 nuclease does not. Also, T2 RNase cleaves at positions A35 and U33 in the anticodon loop whereas S1 cleaves at A36 and A35. Both enzymes give nearly identical patterns when *T.thermophila* 5S rRNA is the substrate, and both cleave only at non-paired bases in a sequence independent manner.

These results demonstrate both the high degree of single-strand specificity of T2 RNase, and in general the probe independent nature of the structure data obtained with the two enzymes, which differ considerably with regard to hydrolytic mechanism(7,13,14).

The concentration of available magnesium ions in RNA samples was reduced by denaturing RNA samples in the absence of cations, followed by renaturation in the presence of Na₂EDTA prior to RNase digestion. Renaturation was performed in a constant background ionic composition of 20mM tris·HCl(pH 7.0) and 100mM NaCl, effecting the competitive removal of Mg²⁺ from the tRNA with excess EDTA. This technique reduces the possibility of RNA degradation compared to standard dialysis methods for Mg²⁺ ion removal. We expect to remove the majority of Mg²⁺ ions from the RNA by this method, leaving only those bound with affinities for the RNA in excess of 100-1000 fold greater than for the chelating agent, EDTA. Changes in the T2 RNase sensitivity of tRNA^{Phe}, therefore, reflect a conformational shift in the population of tRNA^{Phe} molecules as a result of reduction of the concentration of available Mg²⁺ ions. While 5S rRNA showed small shifts in relative rates of hydrolysis of certain phosphodiester bonds upon chelation of Mg²⁺, tRNA^{Phe} showed a dramatic reduction in the sensitivity of the anticodon loop to the single-strand specific T2 RNase. The decrease in sensitivity observed was accompanied by a distinct increase in the sensitivities of the dihydrouracil and the TψC loops as is shown in figures 1 and 2. Previous studies have shown that chelation of divalent cations increases T2 RNase activity(14). Therefore, the observed decrease in sensitivity in the anticodon loop can not be ascribed to changes in the activity of the enzyme. We suggest

that Mg^{2+} ion induced differences in anticodon loop sensitivity to T2 RNase reflect a change in the single-strand loop conformation in tRNA^{Phe}.

T2 RNase was also used to probe tRNA^{Phe} conformation following a pH change from 7.0 to 4.5. The most significant difference observed by lowering the pH was a decrease in anticodon loop sensitivity accompanied by a dramatic increase in the sensitivity of nucleotide D16 in the dihydrouracil loop. The pH dependent change in sensitivity is not likely to be due simply to protonation of the dihydrouracil residue(28). These data suggest that neutral pH favors a structure where the anticodon loop is maximally sensitive to T2 RNase, while lower pH favors a structure in which more exposed dihydrouracil and T ψ C loops occur.

The interpretation of the T2 RNase data in terms of changes in RNA structure are supported by several studies on various tRNAs including yeast tRNA^{Phe}. Bina-Stein and Crothers(11) showed a pH dependence of the melting behavior of several tRNAs in the presence or absence of Mg^{2+} ions. A pH dependent structural transition was detected with a midpoint pH value of 5.5. Later studies confirmed the location of the structural change to the dihydrouracil loop region of the molecule(11). Kearns et al.(9) have shown a dependence of the chemical shifts of hydrogen bonded protons in the dihydrouracil stem. Measurement of the pH dependence of these resonances in yeast tRNA^{Phe} revealed a change in conformation of the dihydrouracil loop and stem structures(9), which is in agreement with the data presented above. Controlled rate studies of the T2 RNase sensitivity of the D16 residue as a function of pH at a constant digestion rate should prove useful in establishing an apparent pK for this transition.

The pH and Mg^{2+} dependent changes in T2 RNase sensitivity seen above for yeast tRNA^{Phe} are also consistent with the results of Schrier and Schimmel(20) and Quigley et al.(12) suggesting that Mg^{2+} appears to be involved in stabilization of tertiary interactions. In addition, Johnston and Redfield(21) used proton NMR to detect a Mg^{2+} sensitive resonance which was assigned to the tertiary G19-C56 base pair in yeast tRNA^{Phe}. This proton is presumably stabilized by Mg^{2+} ions coordinated to the phosphate of G19 and nucleotides G20 and A21(12,21). Finally, Wells used Stern-Volmer quenching of the wye base to measure a Mg^{2+} change in the stacking of the anticodon loop(22). The data presented in figures 1-3 suggest that Mg^{2+} ions cause the anticodon loop to occupy a conformation optimal for interaction with the enzyme probes. A mechanism for metal ion induced sensitivity of the anticodon loop is suggested by two observations. First, $FeCl_3$ can restore T2 RNase sensitivity at the anticodon loop as seen in figure 3. This increase is not due to an effect of the

metal ion on the enzyme rate, since Fe^{3+} ions inhibit T2 RNase(17). Second, micromolar concentrations of Fe^{2+} in the presence of DTT cleaves tRNA^{Phe} only at nucleotides within the dihydrouracil loop, specifically at nucleotide positions G18 and G19(23). These nucleotides also correspond to the cleavage site of tRNA^{Phe} by Pb^{2+} ions(24) and to the location of a bound samarium ion which serves as an isomorphic replacement in tRNA^{Phe}(25). Also, $\text{Fe}^{2+/3+}$ -DTT does not appear to cleave the anticodon loop at concentrations that are sufficient to cause cleavage at the DHU loop(23). These observations, taken together, suggest that small shifts in the distance between the T ψ C and dihydrouracil loops caused by Fe^{3+} or Mg^{2+} binding may specifically alter the conformation of the anticodon loop in such a way that it interacts strongly with T2 RNase. Alternatively, weak binding of Mg^{2+} or Fe^{3+} in the anticodon loop region may be required for anticodon loop sensitivity to T2 RNase.

The biological relevance of this work is suggested by reference to several studies. Peattie and Herr(26) detected a conformational change following binding of E.coli tRNA^{Phe} within the 70S ribosome. An increase in sensitivity of guanine residues in the dihydrouracil loop was demonstrated as a result of the binding of E.coli tRNA^{Phe} within the ribosome, suggesting a role for a relaxation of the tRNA^{Phe} tertiary structure during binding of that molecule within the ribosome. Bertram et al.(27) showed that modification of E.coli phe-tRNA^{Phe} with kethoxal, specifically at position G34 in the anticodon loop, causes a conformational change in distant parts of the molecule, involving the dihydrouracil and T ψ C loop regions. Similar results were obtained for yeast tRNA^{Phe} modified with a carbodiimide reagent in the presence and absence of magnesium(28).

The apparent insensitivity of the Tetrahymena thermophila 5S rRNA T2 RNase hydrolysis pattern to chelation of Mg^{2+} ions is surprising. Previous studies(30,31) suggest that 5 to 10 Mg^{2+} ions per molecule bind tightly to Bacillus stearothermophilus, E. coli and yeast 5S ribosomal RNAs. Our results indicate that such sites, if present in T. thermophila 5S rRNA, must bind Mg^{2+} ions with much higher affinity than EDTA, as implied in previous work by Erdmann and co-workers(30,31).

The results presented above indicate that RNA structure data obtained using T2 RNase can supplement data obtained with S1 nuclease on large and small RNA molecules, and strongly supports the use of T2 RNase as an enzymic probe of RNA conformational behavior over broad ranges of pH and ionic composition. For the case of yeast tRNA^{Phe}, T2 RNase digestion data correlate well with a large body of NMR, optical and x-ray information.

ACKNOWLEDGMENTS

We thank Dr. J. Dabrowiak for assistance with the quantitative densitometric method and N. Rich for development of the software used for data analysis. This work was supported by N.I.H. grant GM 31114.

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