Effect of Magnesium Ion on the Structure of the 5S RNA from *Escherichia coli*. An Imino Proton Magnetic Resonance Study of the Helix I, IV, and V Regions of the Molecule†

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ABSTRACT: The imino proton nuclear magnetic resonance spectrum of *Escherichia coli* 5S ribonucleic acid (RNA) changes when the Mg^{2+} ion concentration drops below physiological levels. The transition between the physiological and low magnesium spectral forms of 5S RNA has a midpoint at approximately 0.3 mM Mg^{2+}. Many of the most conspicuous changes observed in the downfield spectrum of 5S RNA as the magnesium concentration is reduced are due to adjustments in the structures of helices I and IV and the disappearance of resonances originating in helix V. The binding of ribosomal protein L25 to 5S RNA in the absence of magnesium stabilizes helix V structures.

The physical properties of nucleic acids in general are strongly dependent on ionic conditions and respond with particular sensitivity to multivalent cation concentration. The 5S ribosomal ribonucleic acid (RNA)[1] from *Escherichia coli* is no exception. The temperature at which its secondary structure melts depends on Mg^{2+} ion concentration [e.g., see Matveev et al. (1982, 1983)]. The equilibrium between its A and B conformers is magnesium concentration dependent (Richards et al., 1973; Lecanidou & Richards, 1975). Furthermore, there exists an optical transition in the 5S RNA of *E. coli* whose midpoint is near room temperature under physiological ionic conditions and which has the unusual property that the high-temperature form of the molecule is stabilized by Mg^{2+} (Kao & Crothers, 1980). Finally, both the nonexchangeable proton NMR spectrum and the downfield, imino proton NMR spectrum of 5S RNA are conspicuously dependent on the Mg^{2+} ion concentration at room temperature (Kime & Moore, 1982, 1983a).

In this paper, we report a study of the effect of Mg^{2+} concentration on the downfield proton NMR spectrum of 5S RNA at 500 MHz at room temperature. The Mg^{2+}-dependent effect detected by NMR is shown to differ qualitatively from the low-temperature, Mg^{2+}-sensitive, transition of Kao and Crothers with which it was once suggested it might be associated (Kime & Moore, 1982). Many of the most conspicuous Mg^{2+}-dependent spectral changes are demonstrated to be due to modest adjustments of the structures of helices I and IV and the disappearance of resonances belonging to helix V. The influence of magnesium on the downfield spectrum of the helix II–helix III part of the molecule has been documented elsewhere (Leontis & Moore, 1986a). [Some of the data presented below have been discussed previously (Moore, 1985; Leontis et al., 1986).]

Materials and Methods

5S RNA. 5S RNA was obtained from the overproducing strain HB101/pKK5-1 (Brosius, 1984) as described previously (Kime & Moore, 1983b).

Fragment 1. Fragment 1 (bases 1–11 and 69–120 of native 5S RNA) is prepared by treating 5S RNA with RNase A (Kime & Moore, 1983a). The RNA is dissolved in 0.1 M KCl, 5 mM $MgCl_2$, and 50 mM Tris–borate, pH 7.8, at a concentration of 1 mg/mL. The solution is made 1–10 μg/mL in RNase A and incubated on ice for 45 min. At the end of the digestion, the RNase is removed by phenol extraction, and the products are fractionated by chromatography on Sephadex G75.

Spectroscopy. Spectra were obtained in the Fourier transform mode using the 500- and 490-MHz spectrometers at the Yale University Chemical Instrumentation Center. Samples were dialyzed into buffers the concentrations of whose components were 105% of the nominal value, and after dialysis, D_2O was added to a final concentration of 5% to enable the spectrometer lock to function. Samples were brought to the concentrations required for spectroscopy by ultrafiltration using Centricons (Amicon). Dioxane at 2 mM was included in each sample to serve as a chemical shift reference. The chemical shift of dioxane was taken as being 3.741 ppm relative to the methyl resonance of 3-(trimethylsilyl)-1-propanesulfonic acid at all temperatures.

Exchangeable proton spectra were taken by using the twin pulse method (Kime & Moore, 1983a). The ADA technique was used to overcome computer word length limitations (Roth et al., 1980). Spectra were taken in 16K or 8K blocks with a spectral width of 12 000–15 000 Hz, and the offset was usually set at 15 ppm.

Results

Dependence of the Downfield Spectrum of 5S RNA on Mg^{2+} Ion Concentration. A set of 5S RNA samples was prepared by dialysis using a series of buffers of constant composition (0.1 M KCl and 5 mM cacodylate, pH 7.2) except for their content of Mg^{2+} ion. The range covered was 0 (2

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[1] Abbreviations: NMR, nuclear magnetic resonance; ADA, alternate delay accumulation; EDTA, ethylenediaminetetraacetic acid; NOE, nuclear Overhauser effect; RNA, ribonucleic acid; RNase, ribonuclease A; ppm, parts per million; Tris, tris(hydroxymethyl)aminomethane.

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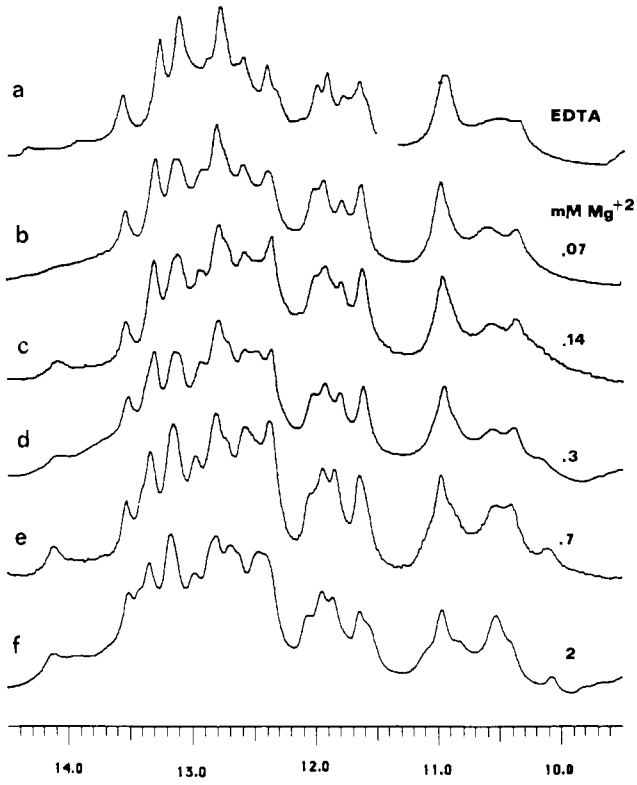

FIGURE 1: Magnesium titration of the 5S RNA imino proton spectrum. A set of 5S RNA samples were prepared by dialysis in buffers containing 0.1 M KCl, 5 mM cacodylate, pH 7.2, and variable magnesium ion concentrations. The RNA concentration was about 1 mM. Downfield spectra were obtained at 303 K as described under Materials and Methods. (a) 5S RNA in 2 mM EDTA; (b) 5S RNA in 0.07 mM $MgCl_2$; (c) 5S RNA in 0.14 mM $MgCl_2$; (d) 5S RNA in 0.30 mM $MgCl_2$; (e) 5S RNS in 0.7 mM $MgCl_2$; (f) 5S RNA in 2 mM $MgCl_2$.

mM EDTA) to 2 mM. The spectra in Figure 1 demonstrate that the imino proton NMR spectrum of 5S RNA depends on Mg^{2+} ion concentration over this range.

Close inspection of the spectra in Figure 1 shows that the change is not the result of a cooperative transition. For example, the sharp resonance, whose chemical shift is about 13.55 ppm at high Mg^{2+} concentration, moves downfield as the Mg^{2+} ion concentration drops, but no change is apparent until the divalent cation concentration gets below 0.14 mM. Its neighbor, a shoulder at 13.45 ppm, shifts upfield and completes its "move" before the Mg^{2+} ion concentration reaches 0.14 mM.

Other experiments have established that 1 mM Mg^{2+} is the upper end of the transition. The spectrum looks the same at 5 mM as it does at 1 mM Mg^{2+}. By the same criterion, the transition's lower bound is about 0.03 mM Mg^{2+}. The midpoint, insofar as it can be defined, occurs around 0.3 mM.

The transition of the spectrum from the low-Mg^{2+} form to the high-Mg^{2+} form is smooth and continuous. At no point is any broadening or doubling of resonances obvious. This behavior suggests that the different conformational states of the molecule involved in this transition are in fast exchange. Since the chemical shift changes of individual resonances during this transition are of the order of 0.1 ppm, maximum (see below), these observations imply that interconversions between conformational states are taking place at rates well in excess of 50 Hz.

Magnesium Effect Is Reversible. Three samples of 5S RNA were prepared by dialysis into 4 mM $MgCl_2$, 0.1 M KCl, and 5 mM cacodylate, pH 7.2. Two of them were then dialyzed into an identical buffer in which the $MgCl_2$ was replaced by 2 mM EDTA, and one of this pair was then dialyzed back into the original, Mg^{2+}-containing buffer. All the dialysis steps were carried out a 4 °C. The two samples in Mg^{2+} had identical downfield spectra, similar to the 2 mM spectrum in Figure 1f, and the sample in EDTA replicated the EDTA spectrum shown in Figure 1a.

To test whether there might be tightly bound magnesium ions stabilizing 5S structure, samples were heated past the molecule's thermodynamic melting point in 2 mM EDTA (N. B. Leontis and J. M. Sturtevant, unpublished results; Matveev et al., 1982, 1983) and then, after being cooled, compared spectroscopically with samples in the same buffer which had not been heat treated. The spectra were identical. (Gels run on these samples showed that heat treatment under these conditions did not lead to degradation.) It is unlikely that tightly bound metal ions contribute to the stabilization of the structure of 5S RNA.

The Mg^{2+} effect on the nonexchangeable proton spectrum can be reversed by direct addition of $MgCl_2$ to magnesium-depleted samples (Kime & Moore, 1982) at room temperature. This result not only shows that the change is reversible for nonexchangeable protons as well as exchangeable ones but also suggests that reversal is fast for both. It takes place in minutes or less, a fact which distinguishes the NMR-detected Mg^{2+} structural change from the A/B transition, which requires hours to days to come to equilibrium at room temperature (Lecanidou & Richards, 1975).

Need for Mg^{2+} during 5S Preparation. The preparative method used for 5S RNA involves steps where the material is deprived of Mg^{2+}. Given that the structure of 5S RNA is sensitive to magnesium, it is reasonable to ask whether the spectra these samples give in the presence of Mg^{2+} are the same as they would be if Mg^{2+} were present at all stages in the purification. To examine this point, a 5S RNA sample was prepared by modifying the normal protocol so that the magnesium concentration was maintained at 5 mM throughout. This sample was indistinguishable spectroscopically and electrophoretically from those prepared in the standard manner (data not shown).

Relationship of the Mg^{2+} NMR Effect to the Kao–Crothers Transition. The Kao–Crothers low-temperature transition in 5S RNA was discovered through optical, temperature-jump experiments (Kao & Crothers, 1980). Several of its properties distinguish it from "normal" nucleic acid optical transitions. First, the form of the molecule stabilized by Mg^{2+} is the one favored by high temperatures. Second, monovalent cations are more effective in influencing the midpoint temperature of the Kao–Crothers transition than divalent cations. Third, the transition is sensitive to pH; high pH favors the high-temperature form of 5S RNA.

A series of experiments was carried out to decide whether the magnesium-dependent, NMR-detected transition is the same as the optical one. In each case, a 5S RNA sample was set at the midpoint of the transition, at 0.3 mM Mg^{2+}, and then environmental factors known to influence the midpoint of the optical transition were varied. Figure 2 shows the result of a heating experiment. The top and bottom spectra are those of intermediate and high magnesium 5S RNA, respectively, at 303 K. Spectra are also shown for the intermediate magnesium sample at 313, 318, and 303 K again following heating. (The similarity of the initial and final spectra taken at 303 K shows that the effects of heating are reversible.) The spectrum of 5S RNA at 0.3 mM Mg^{2+} does not converge to that of the same material in 2 mM Mg^{2+} at 303 K (see Figure

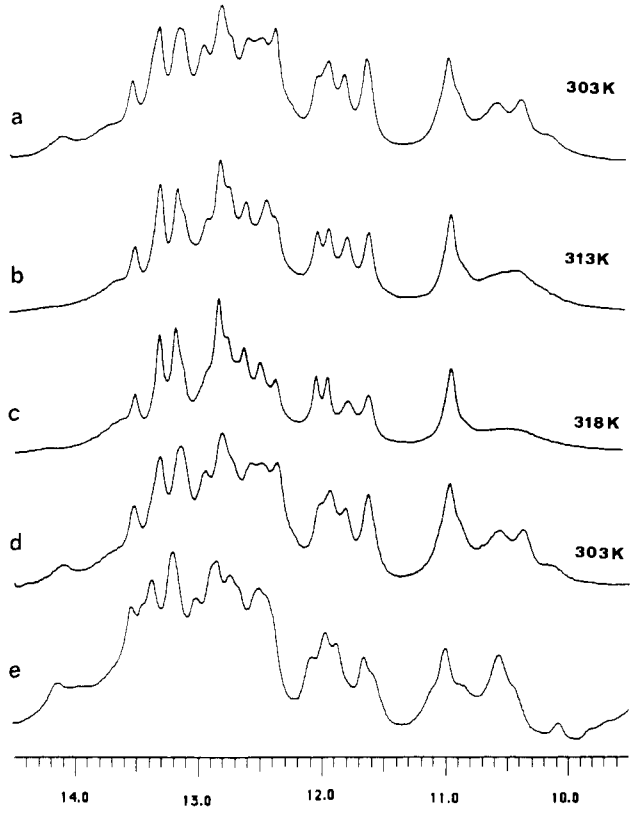

FIGURE 2: Effect of temperature on the downfield spectrum at intermediate magnesium ion concentration. A 1 mM sample of 5S RNA was prepared by dialysis into 0.1 M KCl, 0.3 mM $MgCl_2$, and 5 mM cacodylate, pH 7.2. (a) Downfield spectrum of that sample at 303 K. The next two spectra, (b) and (c), show what was observed at 313 and 318 K, respectively, for that same sample. (d) Spectrum obtained when the sample was returned to 303 K after incubation at 318 K. (e) Spectrum of an otherwise identical sample at 303 K in 2 mM $MgCl_2$.

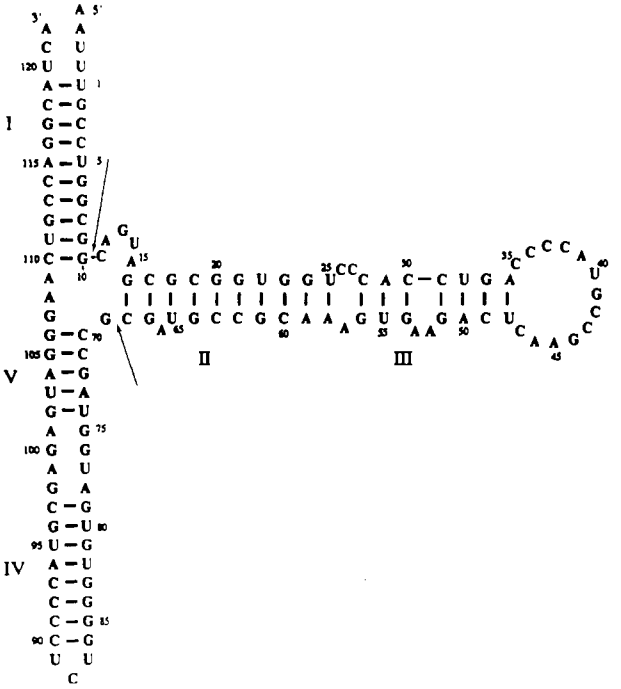

FIGURE 3: 5S RNA of *E. coli*. The sequence of the 5S RNA from the rrnB cistron of *E. coli* is shown in the standard three-stem secondary structure [see Delihas et al. (1984)]. The helical stems are designated with roman numerals for reference purposes. Arrows designate the points where this molecule is cleaved by RNase A to form fragment 1.

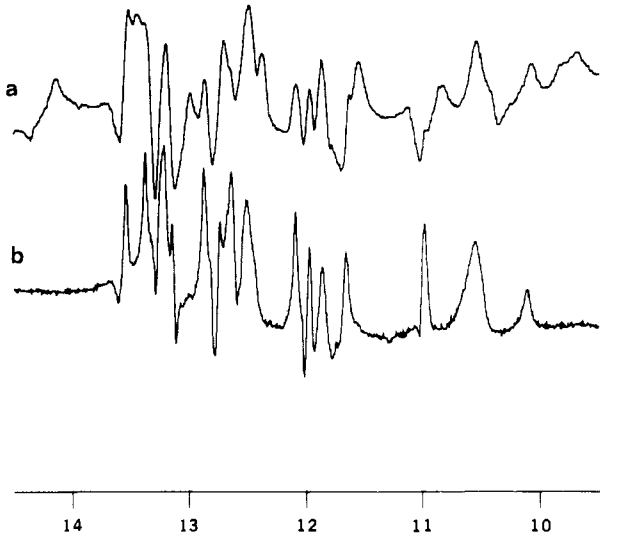

FIGURE 4: Comparison of magnesium difference spectra. The bottom spectrum in this figure, (b), is the difference between a spectrum taken of a 1.5 mM sample of fragment 1 in 0.1 M KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, and 5 mM cacodylate, pH 7.2, at 303 K and that of a similar sample under identical conditions except for the replacement of Mg^{2+} with 2 mM EDTA. The difference is $(+Mg^{2+}) - (-Mg^{2+})$. The top spectrum, (a), is a similar magnesium difference spectrum obtained with samples of intact 5S RNA instead of fragment 1. This 5S difference spectrum has been presented elsewhere (Leontis et al., 1986).

1f) as the temperature is raised, contrary to expectation were the NMR transition the same as the Kao–Crothers transition. The spectrum of 5S RNA in this intermediate stage of the magnesium transition is also unresponsive to added monovalent cation up to 0.5 M (Leontis et al., 1986). In addition, it fails to respond when the pH is taken from 7.2 to 7.9 (data not shown). All three of these environmental perturbations push 5S RNA strongly in the direction of the high-temperature, magnesium-stabilized state characterized by Kao and Crothers. The failure of the NMR spectrum to respond in the same way shows that the events it is reporting are not those responsible for the optical effect.

This finding does not prove that the Kao–Crothers effect has no correlate in the NMR spectrum of 5S RNA, only that its NMR signature, if it exists, is modest compared to the changes observed here.

NMR-Detected, Magnesium-Sensitive Transitions in the Helix I, IV, and V Portions of 5S RNA. The downfield spectrum of 5S RNA is so poorly resolved that it is difficult to interpret its spectral changes. Fortunately, 5S RNA can be "disassembled" into more tractable components by mild nucleolytic digestion (Kime & Moore, 1983a; Leontis & Moore, 1986a), and considerable progress has been made in interpreting the NMR spectra of its domains as isolated entities. The best characterized domain of the molecule is the portion known as fragment 1 which comprises bases 1–11 and bases 69–120 of the intact 5S sequence (Kime & Moore, 1983b,c; Kime et al., 1984; Kime, 1984a,b; Jarema & Moore, 1986). These sequences include helices I, IV, and V of the native molecule (see Figure 3).

Figure 4 compares two difference spectra. The top one, (a), is that observed when the spectrum of intact 5S RNA $(-Mg^{2+})$ is subtracted from that of intact 5S RNA $(+Mg^{2+})$. (It is effectively Figure 1f minus Figure 1a). The bottom spectrum, (b), is the corresponding difference spectrum for fragment 1. [In essence, it is Figure 5a minus Figure 5b (see below).] Positive features in both difference spectra represent intensity

which is observed in the presence of magnesium but not in its absence. Negative features have the converse significance.

Clearly, the downfield spectrum of fragment 1 responds to Mg^{2+} depletion in a manner similar to that of intact 5S RNA. Indeed, many of the most conspicuous alterations seen in the spectrum of 5S RNA upon magnesium depletion are accounted for by alterations observable in the spectrum of fragment 1 under the same conditions. Fragment 1, therefore, is a reasonable model in which to study the magnesium-dependent behavior of the helix I, IV, and V portions of 5S RNA.

There are more positive features than negative features in the fragment 1 difference spectrum (Figure 4b), indicating that the total number of downfield resonances observable decreases when Mg^{2+} ion is removed from the sample. Integrations done on the spectrum of intact 5S RNA have demonstrated that the loss of intensity upon withdrawal of magnesium is 10–15% in the whole molecule (Kime & Moore, 1983a). The balance between positive and negative features in the difference spectrum for intact 5S RNA is less obvious.

Magnesium titration experiments carried out using fragment 1 samples demonstrated that the Mg^{2+}-dependent changes in the fragment 1 spectrum shown in Figure 4 titrate over the same range as was found for intact 5S RNA (data not shown).

Some features of the intact 5S difference spectrum are not accounted for by fragment 1. For example, at 13.45 ppm, the intact 5S difference spectrum shows a positive feature which has no counterpart in the fragment 1 difference signal. A similar discrepancy occurs at 11.5 ppm. That there should be some such features has already been demonstrated in spectroscopic studies of fragment 2 samples as a function of magnesium ion concentration (Leontis & Moore, 1986a). Fragment 2 comprises all of helix III and most of helix II in the native sequence, the part not represented in fragment 1.

Stability of Fragment 1. Initial attempts to characterize the spectrum of fragment 1 in low Mg^{2+} were impeded by the fact that its spectrum changes slowly over many hours incubation at room temperature. Analysis of these samples by gel electrophoresis soon revealed the source of the problem. A large fraction of the molecules in a normal fragment 1 preparation are cleaved at residues 87, 88, and 89. The portion of these cleaved molecules corresponding to bases 69–87 dissociates from the rest of the molecule when the Mg^{2+} concentration is reduced below 0.1 mM. The spectrum given by low-Mg^{2+} samples after long incubations is that of a mixture of dissociated, broken molecules and intact, undissociated ones. The low-Mg^{2+} spectrum of fragment 1 relevant to the structure of intact 5S RNA is, of course, the one given by intact molecules at all times, and by cleaved molecules before dissociation occurs.

In order to characterize the low-magnesium state of fragment 1 spectroscopically, it was essential to obtain a stable preparation. This in turn required that molecules with cleaved loops be separated from the stable, uncleaved molecules. This fractionation turned out to be easy to accomplish. When fragment 1 preparations are chromatographed on Sephadex G100 in 2 mM EDTA, 0.1 M NaCl, and 10 mM cacodylate, pH 6.0 at 30 °C, three species are resolved. The first fraction to elute is the uncleaved molecules. The second is the complex of bases 1–11 and 89–120, and the third peak contains the oligonucleotide corresponding to bases 69–87. [Further details will be published elsewhere (Moore et al., 1987).]

Fractionations of the kind just described were used to remove broken molecules from fragment preparations whose content of unbroken molecules was already maximized by reducing the RNase A concentration during 5S RNA digestion. Intact molecules recovered from this procedure gave normal spectra, following dialysis into Mg^{2+}-containing buffers (Kime et al., 1984). Their electrophoretic mobility in native gels and their L25 binding properties were likewise normal. All the data reported below on fragment 1 in the absence of magnesium were obtained with samples of this kind.

Table I: Chemical Shifts in the Fragment 1 Spectrum in the Presence and Absence of Magnesium Ion at 303 K[a]

resonance, assignment	chemical shifts (ppm) at 303 K: with Mg^{2+}	chemical shifts (ppm) at 303 K: no Mg^{2+}	NOEs obsd, no Mg^{2+}
J, G2–C118	12.58	12.46	
C, C3–G117	13.25	13.19	F,J, 8.57, 6.80
F, C4–G116	12.89	12.82	B,C,E,M, 8.42
B, U5–A115	13.39	13.29	F,M, 6.94 (sharp)
M, G6–C114	12.12	12.02	E
E, G7–C113	13.15	13.13	H,M, 8.57
H, C8–G112	12.74	12.78	E
D, G79–C97	13.22	13.19 (?)	
O, U80–G96	11.89	11.81	Q (strong)
Q1, U80–G96	11.00	11.02	A,O&P (strong)
P1, G81–U95	11.67	11.72	A,Q (strong)
Q2, G81–U95	10.97	11.02	A,O&P (strong)
A, U82–A94	13.53	13.61	N,P.Q, 7.31 (sharp)
N, G83–C93	11.98	11.93	I
I, G84–C92	12.65	12.62	N, 8.50
G	12.82	12.82	
K	12.51		
L	12.46		
R1	10.55	10.55	
R2	10.49	10.45	
S, G75 or G76	10.08		

[a] The samples in question were dissolved in 0.1 M KCl and 5 mM cacodylate, pH 7.4, with either 4 mM $MgCl_2$ or 2 mM EDTA added. Chemical shifts were measured relative to an internal dioxane standard (3.741 ppm). The NOEs listed were observed at temperatures between 293 and 303 K in the absence of magnesium. Aromatic NOEs are listed by their chemical shifts. Comments on NOE intensity or line width are made where appropriate. Assignments for most of the resonances listed may be found elsewhere (Kime & Moore, 1983b,c; Kime et al., 1984; Jarema & Moore, 1986). The correspondence between the spectrum and the resonance nomenclature may be found in Figure 5. The top block of resonances are the helix I resonances in assigned order, reading from the terminal end of the molecule toward its center. The second block are the helix IV resonances again in assigned order, starting from the interior of the molecule and reading toward the 87, 88, 89 loop. The third block of resonances are unassigned or only partially assigned at this time.

Nuclear Overhauser Effects in Fragment 1 in the Absence of Magnesium. Table I summarizes the results of a series of one-dimensional NOE experiments done on the downfield spectrum of fragment 1 at 303 K in the absence of magnesium. Most of the NOE connectivities seen in the presence of the magnesium are also detected in its absence. This fact permits one to equate resonances in spectra taken with Mg^{2+} with those seen in its absence.

Several conclusions emerge. First, helix I which encompasses resonances J, C, F, B, M, E, and H is intact. Second, helix IV's center is preserved (resonances I, N, A, Q, and P). Its proximal end is defective, however. NOEs between resonances O and Q, representing U80–G96, are observed, but the NOE they give to D (G79–C97) in the presence of magnesium is lost. It is not certain that resonance D is represented in the spectrum at all. Furthermore, resonances K, L, and S, all of which are helix V resonances, are absent from the spectrum. Thus, it is clear that base pairing in helix V is weakened by the removal of Mg^{2+}, consistent with the observation that the bases 69–87 portion of fragment 1 slowly dissociates under these conditions. Third, it is clear that the chemical shifts of

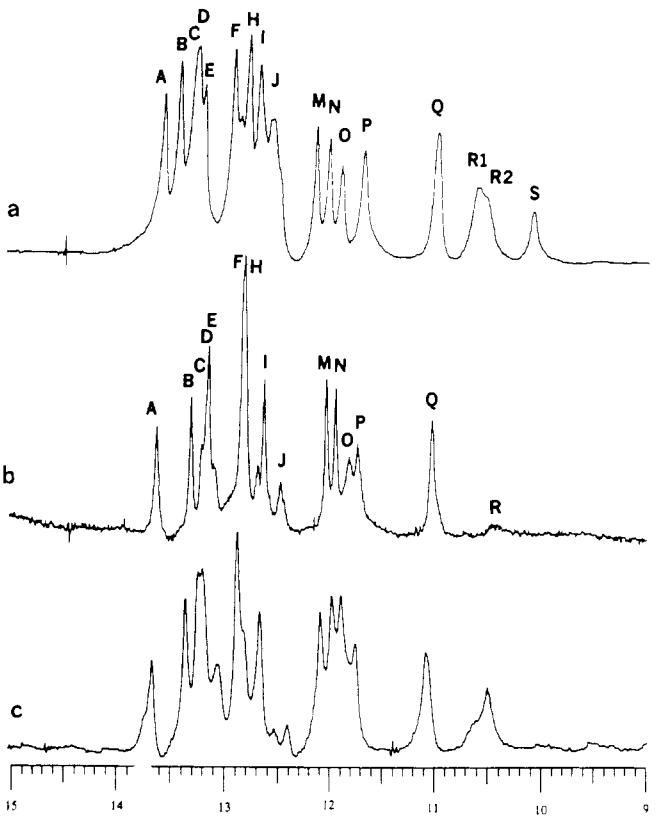

FIGURE 5: Th[illegible]nal response of fragment 1 in low magnesium. A sample of fragment 1 unbroken at the 87, 88, 89 loop was prepared in 0.1 M KCl, 2 mM EDTA, and 0.5 mM Tris, pH 7.5, and its spectrum taken at 303 (b) and 283 K (c). These spectra are compared to that of an ordinary sample of (nicked) fragment 1 in the same buffer except for the replacement of EDTA with 5 mM $MgCl_2$, also at 303 K, spectrum a. Resonance are designated according to the convention of Kime and Moore (1983b). Resonances having the same names in spectra a and b give similar NOEs.

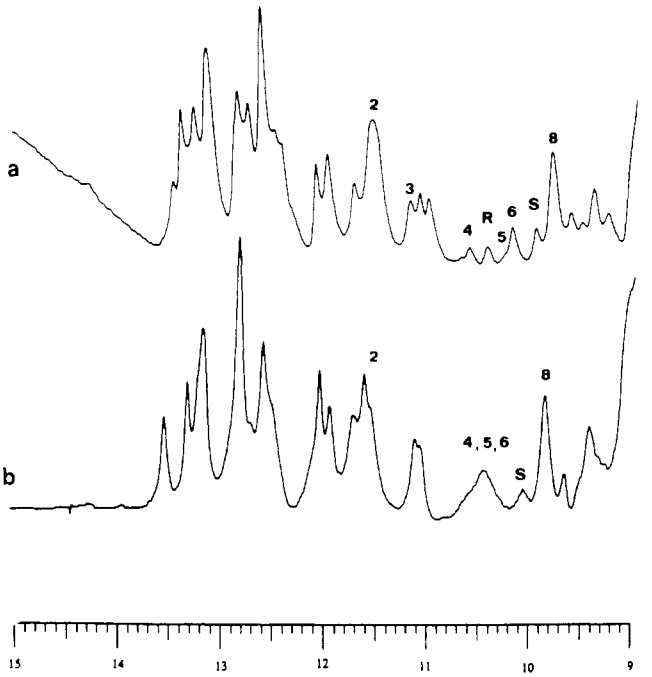

FIGURE 6: Effects of magnesium ion concentration on the downfield spectrum of the fragment 1–L25 complex. Samples of fragment 1–L25 complex were prepared, one in 0.1 M KCl, 5 mM $MgCl_2$, and 5 mM cacodylate, pH 7.2, and the other in 0.1 KCl, 2 mM EDTA, and 0.5 mM Tris, pH 7.5. The spectra given by these two samples at 303 K are spectra a and b, respectively. Numbered resonances indicated in these spectra are resonances unique to the L25 complex (Kime & Moore, 1983b).

virtually all th[illegible]esc[illegible]nces for which comparisons can be made are changed by th[illegible]emoval of Mg^{2+}. The shift changes amount to as much as 0.12 ppm and are in both directions, some upfield and others downfield. Since the spectrum is a superposition of a large number of resonances, its overall appearance is greatly affected by these small shifts through a moire effect. The observer's perception of the extent of change is perhaps out of proportion with what has actually happened to individual resonances.

The spectra given by [illegible]ncleaved samples of fragment 1 in the absence of magnesiu[illegible] did not vary with time. Nevertheless, the covalent structure of the samples used to acquire these data was examined after the experiments were concluded. One denaturing gels, it was apparent that two new strands were being generated at a slow rate, a disappointing finding given the effort made to obtain intact material in the first place. Sequencing showed that these new components were due to cleavage between U103 and A104, which is not observed in the presence of Mg^{2+}. This cleavage could represent a trace contamination of the sample with RNase A. The fact that no cleavage is seen at bases 87, 88, and 89, a region in the molecule highly sensitive to RNase A, argues against this hypothesis, however. Douthwaite and Garrett (1981) have also noted the sensitivity of the U103–A104 bond to "spontaneous" cleavage. Cleavage at U103 does not take place in low Mg^{2+} in the presence of protein L25, a protein known to interact with that region of the molecule (see below).

Fragment 1 in the Absence of Magnesium at Low Temperature. Figure 5 shows spectra for a fragment 1 sample in the absence of magnesium at 303 and 283 K. The resonances in the 303 K spectrum are lettered according to the standard convention (see Table I). At lower temperatures, O and P, which are exchange-broadened at 303 K, sharpen up. The increase in intensity in the N, O, P region coupled with the reappearance of intensity in the R region (10.5 ppm) suggests that the (R2,P) GU base pair is being stabilized. R2,P corresponds to G9–U111 (D. T. Gewirth, S. R. Abo, N. B. Leontis, and P. B. Moore, unpublished results) and is observable only at low temperatures even in the presence of magnesium, both in fragment 1 and in intact 5S RNA (Kime & Moore, 1983b,c). Thus, the situation at the proximal end of helix I in the absence of magnesium is only slightly different from what it is in its presence. The increase in intensity in the C, E region suggests that resonance D is becoming visible again, indicating that the proximal end of helix IV is returning to its high-magnesium state as the temperature is reduced. There is an unidentified resonance between E and F which becomes prominent under these conditions. S, K, and L, however, remain absent. The destabilization of helix V is too great to permit it to be reestablished even by a 20 °C reduction in temperature.

Effect of L25 Binding on the Structure of Fragment 1 in the Absence of Magnesium. Binding between L25 and 5S RNA or fragment 1 is easy to demonstrate in the absence of magnesium ion by gel techniques (Douthwaite et al., 1979; Kime & Moore, 1982). Since this interaction causes marked, specific changes in the downfield spectrum of these RNAs in the presence of magnesium (Kime & Moore, 1983c), it is of interest to examine what happens in the absence of that cation. The downfield spectra of the fragment 1–L25 complex in the presence and absence of magnesium are compared in Figure 6a,b. Table II lists the chemical shifts of resonances in the fragment 1 spectrum in the presence and absence of L25, with and without magnesium.

Table II: Chemical Shifts (ppm) in the Fragment 1 Spectrum in the Presence and Absence of L25, with and without Added Magnesium Ion[a]

	with Mg^{2+} (4 mM)		without Mg^{2+}	
resonance	free RNA	+L25	free RNA	+L25
J	12.58	12.61	12.46	12.56
C	13.25	13.26	13.19	13.19
F	12.89	12.88	12.82	12.79
B	13.39	13.39	13.29	13.28
M	12.12	12.11	12.02	12.02
E	13.15	13.14	13.13	13.13
H	12.74	12.74	12.78	12.79
D	13.22	13.13	13.19	13.13
O	11.89	11.75	11.81	11.70
Q1	11.00	11.12	11.02	11.12 (?)
P1	11.67	11.60	11.72	11.59
Q2	10.97	11.04	11.02	11.06
A	13.53	13.47	13.61	13.50
N	11.98	12.00	11.93	11.92
I	12.65	12.61	12.62	12.56
G	12.82	12.84	12.82	12.79
K	12.51	12.49		12.46
L	12.46	12.43		12.46
R1	10.55	10.50 (?)	10.55	10.45 (?)
R2	10.49	10.50	10.45	10.45 (?)
S	10.08	10.01		10.07
1		12.32		
2		11.52		11.53
3		11.22		11.12 (?)
4		10.66		10.58 (?)
5		10.31		
6		10.25		10.35 (?)
8		9.84		9.86

[a] All samples were dissolved in 0.1 M KCl and 5 mM cacodylate, pH 7.2, with either 4 mM $MgCl_2$ or 2 mM EDTA present. Numbered resonances are resonances specific to the L25 complex. Question marks after a resonance imply that the assignment has not been confirmed by NOE or other methods. See the legend of Table I for further information.

Several conclusions emerge from Table II. First, resonances which belong to helix I (J, C, F, B, M, E, and H) change in chemical shift very little upon L25 binding; 0.01 ppm on the average in the presence of magnesium and 0.02 ppm in its absence. [Three other resonances now known to be helix I resonances, R2, P2, and T (D. T. Gewirth, S. R. Abo, N. B. Leontis, and P. B. Moore, unpublished results), also do not respond to L25 binding (Kime & Moore, 1983c).] The precision we obtain in comparing chemical shifts between spectra taken on different samples under the same ionic conditions and temperature, at different times, is about 0.01 ppm. Thus, the shift changes seen are consistent with the view that the structure being reported by these resonances is unaffected by L25 binding.

With the exception of resonance N, helix IV resonances (I, N, A, P, Q, O, and D), on the other hand, are strongly affected by L25 binding. The average chemical shift change of these resonances is 0.09 ppm in the presence of magnesium and 0.06 ppm in its absence. L25 clearly perturbs the structure of helix IV, both in the presence of magnesium, as reported previously (Kime & Moore, 1983c), and in its absence. A simple explanation for this finding would be that helix IV is part of the L25 binding site under both ionic conditions. Helix IV is known to be included in the region protected from enzymatic attack by L25 in the presence of Mg^{2+} (Garrett et al., 1984; Huber & Wool, 1984).

In addition to perturbing the chemical shifts of helix IV resonances, L25 binding causes a number of new resonances to appear in the downfield part of the spectrum both in the presence of magnesium (Kime & Moore, 1983c) and in its absence (Figure 6). Not all the L25-specific resonances seen in magnesium are evident in its absence, however. Only resonances 2 and 8 are clearly visible in the spectrum of the complex in the absence of Mg^{2+}, and there is a broad feature in the region of resonances 4, 5, and 6. Resonance S, which is not visible in fragment 1 spectra in the absence of magnesium, reappears when L25 is added.

Discussion

It is interesting that in no case did we observe evidence for the presence of the B form of 5S RNA in our samples. B form has a downfield spectrum different from that of the low- and high-magnesium conformations of the molecule (Kime & Moore, 1983a), and its presence in A-form preparations is easily detected on nondenaturing acrylamide gels (Aubert et al., 1968). Richards and his co-workers have reported extensive studies on the A/B equilibrium and the kinetics of this interconversion (Richards et al., 1973; Lecanidou & Richards, 1975), and it is clear that in the absence of Mg^{2+}, especially at elevated temperatures, some B-form material should have appeared during our experiments. It did not.

We have tried to prepare B-form 5S RNA deliberately using rrnB 5S RNA (P. Ghosh, unpublished results). Protocols which we have used successfully in the past on 5S RNA derived from ribosomes (Kime & Moore, 1983a) failed to produce B form from this material. It is our impression that while 5S RNA prepared from ribosomes will go into the B form, the rrnB product will not.

The rrnB 5S sequence differs from the published sequence for 5S RNA at position 12 where an A in the rrnB sequence is replaced by a C (Brosius et al., 1981). There may be something unusual about this part of the rrnB 5S RNA's structure. Fragment 1 prepared from ribosomal 5S RNA has a strand termination after base C11 (Douthwaite et al., 1979). The termination point in rrnB 5S RNA is after G10 (Kime et al., 1984). The only other difference between the overproduced product and the consensus 5S sequence is that the overproduced product includes a few extra bases at both its 3′ and 5′ ends (Kime et al., 1984).

The existence of a magnesium effect and the fact of its reversibility have both been demonstrated with normal, ribosomal 5S RNA. We do not feel that the data or the conclusions offered here about the magnesium effect are invalidated by our use of rrnB 5S RNA. The fact that the rrnB molecule resists going into the B conformation may have fortuitously spared us the taks of separating the magnesium effect from the A/B transition.

There is reason to believe that the B conformation involves base pairing between bases in the region of G41 and nucleotides in the neighborhood of U80 (Trifonov & Bolshoi, 1983; Christensen et al., 1985). The formation of the B structure from the A conformer requires that the base pairing in the helix IV, V region of the native structure be entirely rearranged. It is reasonable to suggest that the loss of helix V structure seen in this study in low magnesium is a step along this pathway.

In fragment 1 samples, it is clear that the oligonucleotide running from base 69 to base 87 is able to dissociate from the rest of the molecule once helix V is destabilized. The slowness of this dissociation is reminiscent of the slowness of the A to B transition under similar conditions (Lecanidou & Richards, 1975).

Garrett and his colleagues have explored the low-magnesium form of 5S RNA by enzymatic probing (Christensen et al., 1985). Their data show that helix V sequences which resist enzymatic attack in the presence of magnesium become sen-

sitive when the concentration of that cation is reduced, consistent with observations reported here. Both the Garrett group and Rabin and co-workers (Rabin et al., 1983) have noted a desensitization of helix IV and the 87, 88, 89 loop to enzymatic attack upon reduction in magnesium concentration. Our data provide no insight into this finding since they appear to indicate only modest perturbations in the helix IV portion of the structure. It has been suggested that a triple helix may form involving this part of fragment 1 and the sequence between C35 and C42 (Christensen et al., 1985), explaining the protection of both regions from enzymatic attack in the low-magnesium structure. The chemical shift changes we observe in helix IV cannot be the result of such an event since they are seen both in intact 5S RNA and in fragment 1 which lacks the C35–C42 sequence altogether.

The most convenient resonance to observe in both fragment 1 and intact 5S RNA which originates in helix V is resonance S. The data now in hand identify S as a GN1 imino proton which is either G75 or G76 (Kime, 1984; Jarema & Moore, 1986; D. T. Gewirth, S. R. Abo, N. B. Leontis, and P. B. Moore, unpublished results). The chemical shift of S, like that of several other helix V associated resonances, is altered when L25 binds to 5S RNA or fragment, showing that the protein interacts with helix V as well as helix IV. The fact that in the absence of magnesium resonance S is not visible in the spectrum of fragment 1 unless L25 is bound provides evidence that L25 stabilizes helix V. Presumably, L25 interacts preferentially with the normal, A form of the helix IV, V structure. Thus, its presence in solutions where the associated, helical form of the helix IV, helix V structure is destabilized tends to drive the equilibrium back toward the A conformation.

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