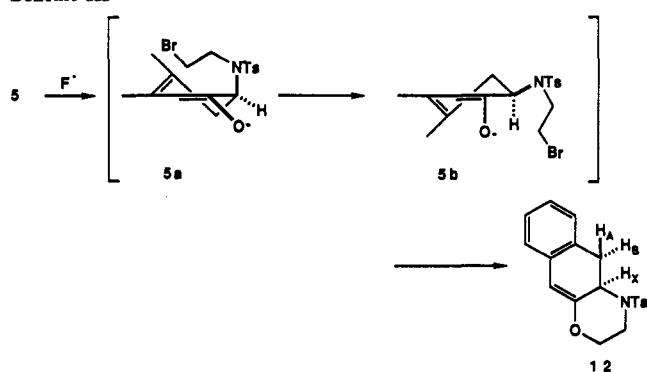


Scheme III



$\beta$ -Tetralone was converted into the triisopropylsilyl enol ether **3** (94%) by treatment with KHMDS/*i*-Pr<sub>3</sub>SiCl/THF/0 °C. Exposure of **3** to (TsN)<sub>2</sub>Se at 25 °C for 40 h gave the axially aminated adduct **4** (71%). Remarkably, this reaction did not result in any aromatization products, which suggests that there is little or no charge buildup in the "ene"/[2.3] sigmatropic rearrangement process. The NHTs group was assigned an axial (pseudo)<sup>5</sup> configuration on the basis of the methine couplings (ABX,  $J_{AX} = 6.0$  Hz,  $J_{BX} = 6.0$  Hz). Treatment of **4** with NaH/BrCH<sub>2</sub>CH<sub>2</sub>Br/THF/80 °C gave the N-alkylated compound **5** (84%), which was directly converted into the sulfide **6** (NaSPh/THF/80 °C (94%). When the derived sulfoxides **7** (MCPBA/CH<sub>2</sub>CH<sub>2</sub>/-78 °C (97%) were treated with trifluoroacetic acid anhydride/2,6-di-*tert*-butyl-4-methylpyridine/CH<sub>2</sub>Cl<sub>2</sub>/0 °C,<sup>6</sup> followed by addition of chlorobenzene and rapid heating to 130 °C, the benzomorphanone **8** was isolated in 50% yield (Scheme II).

The overall structure of **8** and the stereochemistry of the SPh substituent were determined by single-crystal X-ray crystallography.<sup>7</sup> The sulfonium ion **9** is ideally aligned with respect to the  $\pi$ -system of the triisopropylsilyl enol ether to give the oxonium ion **10**. For the case **9** (R = H), only the axial-SPh (synclinal attack) diastereomer was formed. This stereochemical outcome appears to be a consequence of aligning the =SPh<sup>+</sup> group away from the benzo portion of **9** (R = H). Removal of the SPh and Ts groups and concomitant N-methylation of **8** to give **11** (60%) were accomplished by treatment of **8** with Na/NH<sub>3</sub>/THF, followed by methyl iodide (quenching with NH<sub>4</sub>Cl gave the N-nor analogue, 59%).

To further demonstrate conformational immobilization of **4**-**7**, we treated **5** with Bu<sub>4</sub>N<sup>+</sup>F<sup>-</sup>/THF/25 °C and isolated the O-alkylated derivative **12** (88%). The methine proton H<sub>x</sub> is now in an axial configuration ( $J_{AX} = 15.0$  Hz,  $J_{BX} = 6.3$  Hz). Fluoride ion desilylation of **5** gives the enolate **5a**, which is now able to conformationally relax to the equatorial conformation **5b** more rapidly than undergo C-alkylation.<sup>8</sup> Once **5b** is formed it can only undergo O-alkylation resulting in **12** since the geometry of **5b** does not permit C-alkylation (Scheme III).

Starting with the 1-allyl derivative of  $\beta$ -tetralone, its triisopropylsilyl enol ether derivative **13** (97%) was converted into **14** (59%), **15** (87%), **16** (87%), and **17** (99%) as described for **3**.

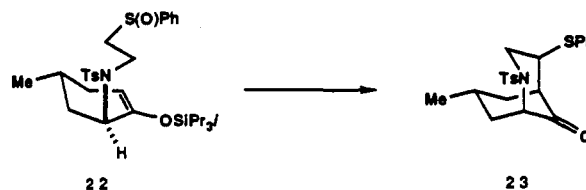
(5) In 1,3-cyclohexadienes it is a moot point as to whether or not an allylic substituent can be designated as axial or even pseudoaxial. Rabideau, P. W.; Sygula, A. Conformational Analysis of 1,3-Cyclohexadienes and Related Hydroaromatics. In *The Conformational Analysis of Cyclohexenes, Cyclohexadienes, and Related Hydroaromatic Compounds*; Rabideau, P. W., Ed.; VCH Inc.: Weinheim, 1989; Chapter 4, p 89.

(6) Gallagher, T.; Magnus, P.; Huffman, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 4750.

(7) Complete details of the single-crystal X-ray crystallographic structure determination of **8** are available from Dr. John C. Huffman (Molecular Structure Center, Indiana University, Bloomington, IN 47405). Please ask for Report No. 90901.

(8) We cannot exclude the possibility that the axial conformer **5a** undergoes exclusive O-alkylation to give, after conformational relaxation, **12**. In view of the pronounced tendency for cyclohexanone formation (Baldwin, J. E.; Kruse, L. I. *J. Chem. Soc., Chem. Commun.* **1977**, 77), this alternative seems less likely.

When **17** was exposed to the Pummerer reaction conditions (TFAA/2,6-di-*tert*-butyl-4-methylpyridine/CH<sub>2</sub>Cl<sub>2</sub> at 0 °C and then PhCl at 130 °C, the benzomorphanone adduct **18** was isolated as a mixture of epimers (1.7:1, 79% yield) at the C-SPh bond. Treatment of **18** with Na/NH<sub>3</sub>/THF, followed by methyl iodide, gave **21** (57%). This method for making azabicyclo[3.3.1] systems is equally applicable to simple cyclohexane derivatives. For example, when **22** was exposed to the above Pummerer-type conditions, the 2-azabicyclo[3.3.1]nonan-9-one **23** was isolated in 54% yield.



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**Supplementary Material Available:** General spectral details for compounds **4**-**6**, **8**, **11**-**16**, **18**, **21**, and **23**, details of the X-ray structure determination of **8**, and tables of fractional coordinates, isotropic thermal parameters, anisotropic thermal parameters, bond lengths, and bond angles for C<sub>25</sub>H<sub>23</sub>S<sub>2</sub>O<sub>3</sub>N (21 pages). Ordering information is given on any current masthead page.

### Metallobiochemistry of a Ribosomal RNA. A Possible Role for Na<sup>+</sup> and K<sup>+</sup> in the Regulation of Mg<sup>2+</sup> Binding Sites on *Escherichia coli* 5S rRNA: Implications for Activity

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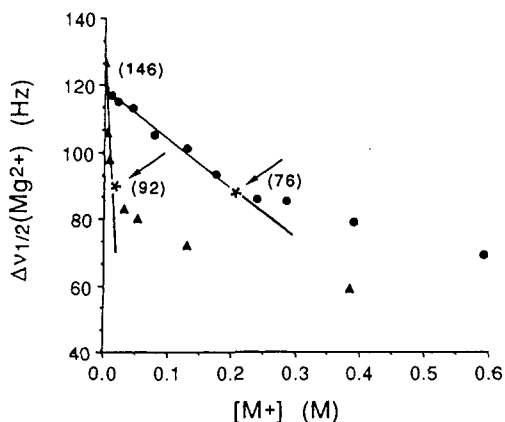
Although alkali and alkaline-earth metal ions are essential cofactors in the structural and catalytic chemistry of RNA,<sup>1-5</sup> there

(1) Latham, J. A.; Cech, T. R. *Science* **1989**, *245*, 276. Been, M. D.; Cech, T. R. *Science* **1988**, *239*, 1412. Altman, S.; Guerrier-Takada, C.; Frankfort, H. M.; Robertson, H. D. In *Nucleases*; Linn, S. M., Roberts, R. J., Eds.; Cold Spring Harbor Laboratory: 1985; pp 243 and 311.

(2) Moore, P. B. *Nature* **1988**, *331*, 223-227. Tang, R. S.; Draper, D. E. *Biochemistry* **1990**, *29*, 5232-5237. Ryan, P. C.; Draper, D. E. *Biochemistry* **1989**, *28*, 9949-9956. Wittmann, H. G. *Annu. Rev. Biochem.* **1982**, *51*, 155-183.

(3) Leontis, N. B.; Moore, P. B. *Biochemistry* **1986**, *25*, 5736-5744. Leontis, N. B.; Ghosh, P.; Moore, P. B. *Biochemistry* **1986**, *25*, 7386-7392. Kime, M. J.; Ratcliffe, R. G.; Moore, P. B.; Williams, R. J. P. *Eur. J. Biochem.* **1981**, *116*, 169-276. Kime, M. J.; Moore, P. B. *Nucleic Acids Res.* **1982**, *10*, 4973-4983.

(4) A large body of literature exists on DNA-cation interactions, e.g.: Record, M. T.; Woodbury, C. P.; Lohman, T. M. *Biopolymers* **1976**, *15*, 893-915. Record, M. T. *Biopolymers* **1975**, *14*, 2137-2138. Record, M. T.; Lohman, T. M.; de Haseth, P. *J. Mol. Biol.* **1976**, *107*, 145-158. Manning, G. S. *Biopolymers* **1972**, *11*, 937-949. Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 924-938. Krakauer, H. *Biopolymers* **1971**, *10*, 2459-2490. Krakauer, H. *Biochemistry* **1974**, *13*, 2579-2589. However, these do not account for the more complex tertiary structure of RNA relative to DNA, while the theoretical analyses were based on considerations of the electrostatic interaction of cations with a phosphate backbone, and do not account for the possibility of H-bonding interactions with nucleotide bases and sugar hydroxyls. Further work in this area is therefore called for.



**Figure 1.** Variation of  $\Delta\nu_{1/2}(\text{Mg}^{2+})$  with concentration of monovalent cation  $[\text{M}^+]$  for a  $63 \mu\text{M}$  solution of 5S rRNA (in  $1800 \mu\text{L}$  of  $\text{H}_2\text{O}$ ,  $100 \mu\text{L}$  of  $\text{D}_2\text{O}$ , pH 6.8, containing  $18.5 \text{ mM } ^{25}\text{Mg}^{2+}$ ) following titration with  $\text{Na}^+$  ( $\blacktriangle$ ) and  $\text{K}^+$  ( $\bullet$ ). Error bars are not shown for  $\Delta\nu_{1/2}(\text{Mg}^{2+})$  but are of the order of  $\pm 5\%$ . Typical spectral parameters were as follows: spectral digitization, SW = 10000 Hz, SI = 1 K, TD = 1 K; pulse width =  $90^\circ$  ( $30 \mu\text{s}$ ); preacquisition delay =  $200 \mu\text{s}$ ; the spectrum was zero-filled. (\*) denotes where the plots change curvature. The number of available binding sites for  $\text{Mg}^{2+}$  ions in parentheses at critical points on the figure (no added cations,  $n = 146$ ;  $12 \text{ mM Na}^+$ ,  $n = 92$ ;  $180 \text{ mM K}^+$ ,  $n = 76$ ). These were determined by procedures described in the text that were independent of the NMR measurements. The data is, however, in full agreement with the NMR results.

is a general lack of information on the number of binding sites available to these metal ions and in the understanding of their competitive binding chemistry. In this paper we describe a simple procedure for determining the number of bound  $\text{Mg}^{2+}$  ions  $[n; \text{RNA}(\text{Mg}^{2+})_n]$  under different solution conditions and demonstrate the use of  $^{25}\text{Mg}$  NMR to monitor the dependence of  $n$  on the concentration of  $\text{Na}^+(\text{aq})$  and  $\text{K}^+(\text{aq})$ . Together these represent a general protocol for studying the metallobiochemistry of important RNA complexes. We document the analysis of 5S rRNA (*Escherichia coli*) as a specific example and present evidence for two classes of  $\text{Mg}^{2+}$  binding sites on this molecule.<sup>6</sup>

The value of  $n$  in  $\text{RNA}(\text{Mg}^{2+})_n$  was determined from the effective concentration of bound ion ( $[\text{Mg}^{2+}]_{\text{bound}}$ ) when excess  $\text{Mg}^{2+}$  was added to a solution containing a defined amount of 5S rRNA. The ratio  $[\text{Mg}^{2+}]_{\text{bound}}/[\text{RNA}]$  gave the total number of sites:  $[\text{Mg}^{2+}]_{\text{bound}} = [\text{Mg}^{2+}]_{\text{total}} - [\text{Mg}^{2+}]_{\text{free}}$ . Specifically: 5 mg of 5S rRNA was dissolved in  $400 \mu\text{L}$  of  $\text{H}_2\text{O}$ , containing known  $[\text{Mg}^{2+}]$ ,  $[\text{K}^+]$ , or  $[\text{Na}^+]$ , and stored at  $298 \text{ K}$  for 1 h. Use of greater amounts of  $\text{Mg}^{2+}$  gave similar results; therefore the binding sites were saturated under the conditions employed ( $500 \text{ equiv Mg}^{2+}$ ). Two independent methods were used to isolate free  $\text{Mg}^{2+}(\text{aq})$  from bound  $\text{Mg}^{2+}$ : first, the separation of free  $\text{Mg}^{2+}(\text{aq})$  and complexed  $\text{RNA}(\text{Mg}^{2+})_n$  by ultrafiltration (centricon 30;  $40 \text{ min}$ );<sup>7</sup> or second, rapid precipitation and pelleting (Eppendorf) of the  $\text{RNA}(\text{Mg}^{2+})_n$  complex ( $\text{EtOH}$ ,  $-20^\circ\text{C}$ ).  $[\text{Mg}^{2+}]_{\text{bound}}$  was determined from  $[\text{Mg}^{2+}]_{\text{total}} - [\text{Mg}^{2+}]_{\text{free}}$  by measuring the concentrations of each of the latter by atomic absorption (AA).<sup>8,9</sup> The precipitation

procedure is experimentally more convenient, but in either case ca.  $146 \pm 20 \text{ Mg}^{2+}$  ions bind to 5S rRNA in the absence of background salt.<sup>10</sup> The value of  $[\text{Mg}^{2+}]_{\text{bound}}$  in the isolated  $\text{RNA}(\text{Mg}^{2+})_n$  pellet can also be determined directly from AA measurements by redissolving the complex. The results are in accord with those determined above. It is assumed that there is no significant dissociation of bound  $\text{Mg}^{2+}$  during the isolation procedure, and this assumption is fully justified by the close agreement obtained with each distinct method.

Addition of either excess  $\text{Na}^+$  or  $\text{K}^+$  resulted in the displacement of 40–50% of the bound  $\text{Mg}^{2+}$  ions (determined by the procedures noted above) and was readily monitored by use of  $^{25}\text{Mg}$  NMR.<sup>9,11–14</sup> The variation of  $^{25}\text{Mg}$  line width  $[\Delta\nu_{1/2}(\text{Mg}^{2+})]$  with concentration of metal ion (Figure 1) suggests that the  $\text{Mg}^{2+}$  binding sites fall into two categories: one where  $\text{Mg}^{2+}$  is readily displaced by  $\text{Na}^+$  or  $\text{K}^+$ , and a second class that is less readily substituted by monovalent cations. The decrease in  $\Delta\nu_{1/2}(\text{Mg}^{2+})$  with increasing  $[\text{K}^+]$  is biphasic with a break point at  $[\text{K}^+] \sim 180 \text{ mM}$ .<sup>13</sup> A similar titration with  $\text{Na}^+$  produced a dramatic reduction in  $\text{Mg}^{2+}$  binding up to  $[\text{Na}^+] \sim 12 \text{ mM}$ , followed by a shallower decay that paralleled the results for the later stages of the  $\text{K}^+$  titration. For both  $\text{Na}^+$  and  $\text{K}^+$ ,  $\Delta\nu_{1/2}(\text{Mg}^{2+})$  at the break point [denoted by (\*) in Figure 1] was similar and, within experimental error, an equivalent number of  $\text{Mg}^{2+}$  ions (ca. 40% of the total number of sites, ref 14) were displaced, suggesting that the monovalent cations are competing for the same  $\text{Mg}^{2+}$  binding sites. The greater effectiveness of  $\text{Na}^+$  as a substitute for  $\text{Mg}^{2+}$  reflects the relative ionic radii of the three ions ( $\text{Mg}^{2+}$ ,  $0.65 \text{ \AA}$ ;  $\text{Na}^+$ ,  $0.95 \text{ \AA}$ ; relative to  $\text{K}^+$ ,  $1.33 \text{ \AA}$ ). Control experiments with small phosphate ligands (e.g.,  $\text{ATP}^{4-}$ ,  $\text{ADP}^{3-}$ , and glucose 1-phosphate) show no dependence of  $\Delta\nu_{1/2}(\text{Mg}^{2+})$  on  $[\text{K}^+]$  or  $[\text{Na}^+]$  in the concentration range used.

The plots in Figure 1 can be explained by competitive displacement of  $\text{Mg}^{2+}$  from rRNA by  $\text{Na}^+(\text{aq})$  and  $\text{K}^+(\text{aq})$ . The break point (\*) in the plot of  $\Delta\nu_{1/2}(\text{Mg}^{2+})$  with  $[\text{Na}^+]$  or  $[\text{K}^+]$  (Figure 1) suggests two classes of  $\text{Mg}^{2+}$  binding sites and correlates remarkably well with the intracellular levels of these ions ( $5\text{--}15$  and  $140 \text{ mM}$ , respectively),<sup>15</sup> and so the change in  $\text{Mg}^{2+}$  binding in the presence of either  $\text{Na}^+(\text{aq})$  or  $\text{K}^+(\text{aq})$  is likely to be of functional significance. Sodium is frequently neglected in discussions of ribosomal chemistry because of the low intracellular concentration of this ion;<sup>15</sup> however, it is clear that  $\text{Na}^+$  can compete effectively with  $\text{K}^+$  under typical physiological conditions.

In this paper we have determined for the first time the number of  $\text{Mg}^{2+}$  ions bound to 5S rRNA under various solution conditions<sup>14</sup> and demonstrated a dependence of  $\text{Mg}^{2+}$  binding on  $[\text{Na}^+]$  and  $[\text{K}^+]$  that closely matches the concentrations of these ions in the cell. The data suggests a regulatory role for alkali-metal ions as triggers of structural change and  $\text{Mg}^{2+}$  binding in cellular RNA.<sup>5,16,17</sup> Previous interpretations of published data may require

(10) Under certain solution conditions, the number of  $(\text{Mg}^{2+})_{\text{bound}}$  ions is comparable to the number of phosphates on the backbone (ca. 120). This is accounted for by outer-sphere complexation of  $\text{Mg}(\text{H}_2\text{O})_6^{2+}$  to O and N atoms on exposed bases and ribose units in dsRNA and ssRNA: Cowan, J. A.; Hsu, L.-Y., in preparation.

(11) Resonances were Lorentzian, and data lies in the near extreme narrowing limit ( $\tau_c\omega_0 \leq 1.5$ ).

(12) Forsen, S.; Lindman, B. *Annu. Rep. NMR Spectrosc.* **1981**, *11A*, 183. Vogel, H. J.; Forsen, S. In *Biological Magnetic Resonance*; Berliner, L. J., Reuben, J., Eds.; Plenum: New York, 1986; Vol. 7, pp 249–307.

(13) These data are distinct from plots for the competitive binding of metal ions on DNA (Rose, D. M.; Bleam, M. L.; Record, M. T.; Bryant, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6289. Bleam, M. L.; Anderson, C. F.; Record, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3085), where there is no break point and the plots level off at a line width that corresponds to free metal ion (i.e., total displacement).

(14) The number of  $\text{Mg}^{2+}$  ions bound to 5S rRNA under various critical solution conditions is as follows: ( $\pm 15\%$  error) no external cations added ( $n = 146$ );  $12 \text{ mM NaCl}$  ( $n = 92$ );  $180 \text{ mM KCl}$  ( $n = 76$ ).<sup>10</sup> Refer to Figure 1.

(15) Raw, J. D. *Biochemistry*; Neil Patterson Publishing Co.: Burlington, 1989; p 1028.

(16) Li, S.-J.; Marshall, A. G. *Biochemistry* **1985**, *24*, 4047–4052. Marshall, A. G.; Wu, J. In *Biological Magnetic Resonance*; Berliner, L. J., Reuben, J., Eds.; Plenum: New York, 1990; Vol. 9, pp 55–118 and references therein.

(5) LeCanidou, R.; Richards, E. G. *Eur. J. Biochem.* **1975**, *57*, 127–133. Richards, E. G.; LeCanidou, R.; Geroch, M. E. *Eur. J. Biochem.* **1973**, *34*, 1262–1267. Kao, T.-H.; Crothers, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3360–3364. Rabin, D.; Kao, T.-H.; Crothers, D. M. *J. Biol. Chem.* **1983**, *258*, 10813–10816.

(6) 5S rRNA was derived from the *rrnB* gene overproduced in *E. coli*/pKK5-1 (Brosius, J.; Dull, T. J.; Sleeter, D. D.; Noller, H. F. *J. Mol. Biol.* **1981**, *148*, 107–127. Kime, M. J.; Moore, P. B. *Biochemistry* **1983**, *22*, 2615–2622) and isolated by following the procedure reported previously for the isolation of *Bacillus subtilis* 5S rRNA: Li, S.-J.; Chang, L.-H.; Chen, S.-M.; Marshall, A. G. *Anal. Biochem.* **1984**, *138*, 465–471.

(7) The ultrafiltration method separated  $\text{RNA}(\text{Mg}^{2+})_n$  from unbound  $\text{Mg}^{2+}$  using centricons (Amicon Ltd.; membrane cutoff,  $30 \text{ kDa}$ ). A correction was made for the small volume of solution left in the filtration device (typically ca.  $20 \mu\text{L}$ ).

(8)  $[\text{Mg}^{2+}]_{\text{total}}$  refers to the total amount of  $\text{Mg}^{2+}$  added to the solution.  $[\text{Mg}^{2+}]_{\text{free}}$  refers to unbound  $\text{Mg}^{2+}(\text{aq})$  that is free in solution.

(9) Reid, S. S.; Cowan, J. A. *Biochemistry* **1990**, *29*, 6025–6032.

reconsideration in the light of known physiological levels of  $Mg^{2+}$  (10–30 mM) and the demonstrated affinity of 5S rRNA for  $Mg^{2+}$ . The methodology described herein should be of general utility in developing the metallobiochemistry of many structurally and catalytically important RNA molecules.

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(17) Leontis, N. B.; Ghosh, P.; Moore, P. B. *Biochemistry* 1986, 25, 7386–7392.

### Coordination Chemistry of $Mg^{2+}$ and 5S rRNA (*Escherichia coli*): Binding Parameters, Ligand Symmetry, and Implications for Activity

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Alkali and alkaline-earth metals are the most abundant cations in living organisms and are essential for the proper regulation of cellular bioenergetics, protein synthesis, and enzymatic chemistry on RNA and DNA.<sup>1,2</sup> However, the absence of convenient physical and spectroscopic properties to study the ligand environment of these ions has held back the detailed understanding of their biochemistry.<sup>3–7</sup> Previously we described simple procedures to determine the number of  $Mg^{2+}$  ions bound to 5S rRNA.<sup>5</sup> In this paper we describe the first detailed quantitative study of the coordination chemistry of  $Mg^{2+}$  with a rRNA. Important binding parameters ( $K_a$ ,  $\Delta G^*$ ,  $k_{off}$ )<sup>6</sup> have been determined by direct measurement. The coordination state (inner/outer sphere) of the magnesium center can be deduced from consideration of the nuclear quadrupole coupling constant  $\chi_B$ , while the energetics of  $Mg^{2+}$ -RNA binding contains a large contribution from hydrogen-bonding interactions of inner-sphere  $H_2O$  molecules to backbone phosphates, sugar hydroxyls, and nucleotide bases.

The coordination chemistry of  $Mg^{2+}$  with 5S rRNA was studied by use of  $^{25}Mg$  NMR, which offers a probe of binding kinetics ( $k_{on}$ ,  $k_{off}$ ,  $\Delta G^*$ ), thermodynamics ( $K_a$ ), and ligand geometry (quadrupole coupling constant  $\chi_B$ ).<sup>6,8</sup> A correlation time  $\tau_c \sim 10$  ns has been previously estimated for 5S rRNA in solution.<sup>9</sup>

(1) Nicholls, D. G. *Bioenergetics*; Academic Press: New York, 1982.  
(2) Moore, P. B. *Nature* 1988, 331, 223–227. *Nucleases*; Linn, S. M., Roberts, R. J., Eds.; Cold Spring Harbor Laboratory: 1985. McClarin, J. A.; Frederick, C. A.; Wang, B.-C.; Greene, P.; Boyer, H. W.; Grable, J.; Rosenberg, J. M. *Science* 1986, 234, 1526.

(3) Recent NMR and theoretical studies have given considerable insight on metal binding to DNA in particular.<sup>4</sup>

(4) Rose, D. M.; Bleam, M. L.; Record, M. T.; Bryant, R. G. *Proc. Natl. Acad. Sci. U.S.A.* 1980, 77, 6289–6292. Bleam, M. L.; Anderson, C. F.; Record, M. T. *Proc. Natl. Acad. Sci. U.S.A.* 1980, 77, 3085–3089. Braunlin, W. H.; Nordenskiöld, L.; Drakenberg, T. *Biopolymers* 1989, 28, 1339–1342. James, T. L.; Noggle, J. H. *Proc. Natl. Acad. Sci. U.S.A.* 62, 644–649. Record, M. T.; Woodbury, C. P.; Lohman, T. M. *Biopolymers* 1976, 15, 893–915. Record, M. T. *Biopolymers* 1975, 14, 2137–2138. Manning, G. S. *Biopolymers* 1972, 11, 937–949. Manning, G. S. *J. Chem. Phys.* 1969, 51, 924–938. Krakauer, H. *Biopolymers* 1971, 10, 2459–2490. Krakauer, H. *Biochemistry*, 1974, 13, 2579–2589.

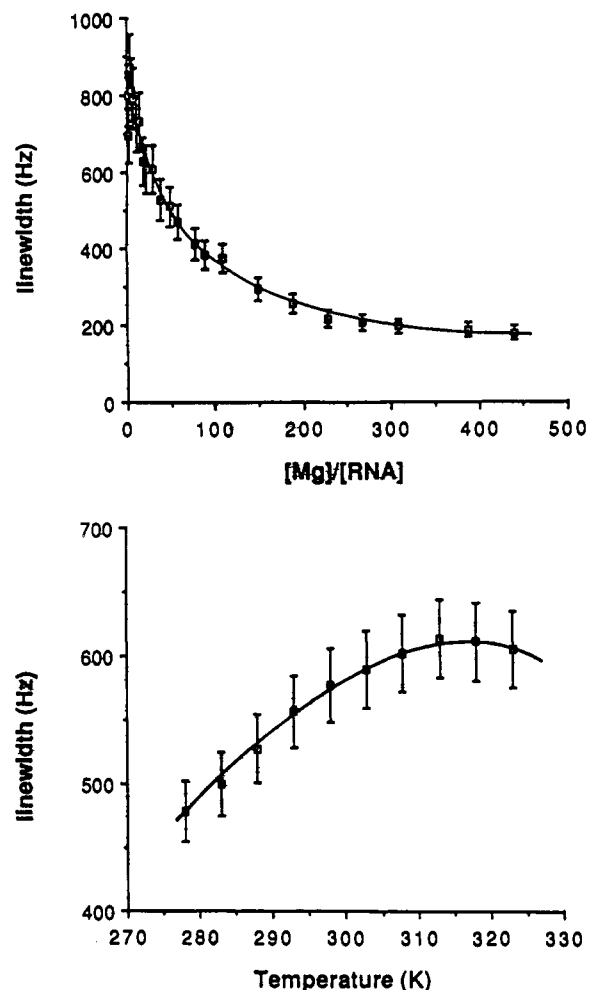
(5) Reid, S. S.; Cowan, J. A., published in this issue.

(6) Forsen, S.; Lindman, B. *Annu. Rep. NMR Spectrosc.* 1981, 11A, 183.

(7) Kao, T.-H.; Crothers, D. M. *Proc. Natl. Acad. Sci. U.S.A.* 1980, 77, 3360–3364. Rabin, D.; Kao, T.-H.; Crothers, D. M. *J. Biol. Chem.* 1983, 258, 10813–10816.

(8) Reid, S. S.; Cowan, J. A. *Biochemistry* 1990, 29, 6025–6032.

(9) Marshall, A. G.; Smith, J. L. *Biochemistry* 1980, 19, 5955.



**Figure 1.** Top: Magnesium titration curve obtained at 298 K in 0.2 M NaCl (pH 7).  $[Mg^{2+}]$  varied from 0.22 to 77 mM,  $[RNA]$  varied from 0.22 to 0.18 mM. Bottom: Variation of line width with temperature.  $[Mg^{2+}] = 7.5$  mM,  $[RNA] = 0.2$  mM in 0.2 M NaCl (pH 7). Typical spectral parameters were as follows: preacquisition delay = 100  $\mu$ s, pulse width =  $90^\circ$  (30  $\mu$ s); (broad lines) SW 20 000 Hz, SI = 512 W, AQ = 26 ms; (narrow lines) SW = 3000 Hz, SI = 512 W, AQ = 171 ms. The experimental points are shown relative to a theoretical curve obtained by joining calculated points from the fitting analysis.  $\Delta v_{1/2}$  included a line broadening of 100 Hz.

If the bound ion were to possess internal rotational freedom [i.e.,  $\tau_c(Mg^{2+})_{bound} < \tau_c(RNA)$ ], the association constant ( $K_a$ ) and off rate ( $k_{off}$ ) would not be affected but  $\chi_B$  might be underestimated. By consideration of the relaxation parameters  $T_1$  and  $T_2$ ,  $\tau_c(Mg^{2+})_{bound}$  was estimated to be ca. 12 ns.<sup>10</sup> This clearly demonstrates the lack of rotational freedom for bound ion, and so exchange falls in the near-extreme narrowing region ( $\omega_0\tau_c \leq 1.5$ ).<sup>11–13</sup> The exchange-broadened resonance is therefore dominated by a single relaxation term and is indistinguishable from a Lorentzian form.<sup>12</sup> In 0.2 M NaCl the native ("high" melting) conformation is adopted,<sup>14,15</sup> and Figure 1 shows the effect on  $^{25}Mg^{2+}$  line width  $[\Delta v_{1/2}(Mg^{2+})]$  when increasing amounts of ion

(10) The value of  $\tau_c$  at a given temperature can be calculated from<sup>11,12</sup>

$$T_2/T_1 = 2 \frac{1/[1 + (\omega_0\tau_c)^2] + 4/[1 + 4(\omega_0\tau_c)^2]}{3 + 5/[1 + (\omega_0\tau_c)^2] + 2/[1 + 4(\omega_0\tau_c)^2]}$$

(11) Vogel, H. J.; Forsen, S. In *Biological Magnetic Resonance*; Berliner, L. J., Reuben, J., Eds.; Plenum: New York, 1986; Vol. 7, pp 249–307. Drakenberg, T.; Forsen, S.; Lilja, H. *J. Magn. Reson.* 1983, 53, 412–422.

(12) Halle, B.; Wennerstrom, H. *J. Magn. Reson.* 1981, 44, 89–100.

(13) Since  $\tau_c(Mg^{2+})_{bound} < \tau_c(RNA)$ , this must hold true even if there exists some internal freedom of motion for  $(Mg^{2+})_{bound}$ .

(14) LeCanidou, R.; Richards, E. G. *Eur. J. Biochem.* 1975, 47, 127–133.

(15) Richards, E. G.; LeCanidou, R.; Geroch, M. E. *Eur. J. Biochem.* 1973, 34, 1262–1267.