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.

Acknowledgment. We thank H. Noller and Kathy Triman (U.C. Santa Cruz) for providing the overproducing *E. coli* strain for 5S rRNA. FT-NMR spectra (300 MHz) were obtained at The Ohio State University Chemical Instrument Center. This work was supported in part by a seed grant from the American Cancer Society, administered by The Ohio State University.

(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

J. A. Cowan

Evans Laboratory of Chemistry, The Ohio State University
120 West 18th Avenue, Columbus, Ohio 43210

Received July 9, 1990

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.[1,2] 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.[3-7] Previously we described simple procedures to determine the number of Mg^{2+} ions bound to 5S rRNA.[5] 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, ΔG^*, k_{off})[6] 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 χ_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}, ΔG^*), thermodynamics (K_a), and ligand geometry (quadrupole coupling constant χ_B).[6,8] A correlation time $\tau_c \sim 10$ ns has been previously estimated for 5S rRNA in solution.[9]

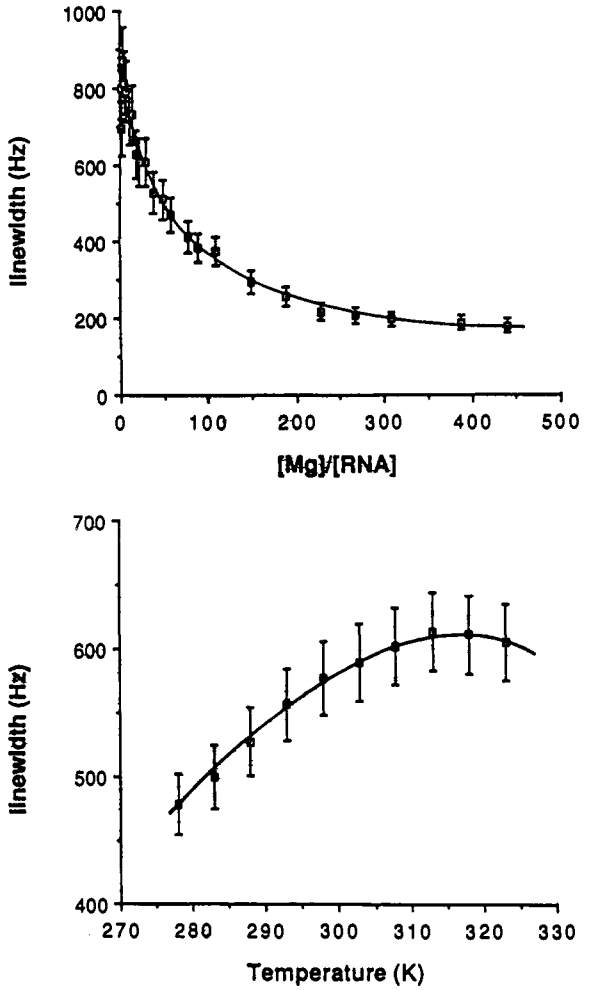

Figure 1. Top: Magnesium titration curve obtained at 298 K in 0.2 M NaCl (pH 7). $[Mg^{2+}]$ varied from 0.22 to 77 mM, [RNA] varied from 0.22 to 0.18 mM. Bottom: Variation of line width with temperature. $[Mg^{2+}]$ = 7.5 mM, [RNA] = 0.2 mM in 0.2 M NaCl (pH 7). Typical spectral parameters were as follows: preacquisition delay = 100 μs, pulse width = 90° (30 μs); (broad lines) SW 20000 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. $\Delta\nu_{1/2}$ 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 χ_B might be underestimated. By consideration of the relaxation parameters T_1 and T_2, τ_c-$(Mg^{2+})_{bound}$ was estimated to be ca. 12 ns.[10] This clearly demonstrates the lack of rotational freedom for bound ion, and so exchange falls in the near-extreme narrowing region ($\omega_o\tau_c \leq 1.5$).[11-13] The exchange-broadened resonance is therefore dominated by a single relaxation term and is indistinguishable from a Lorentzian form.[12] In 0.2 M NaCl the native ("high" melting) conformation is adopted,[14,15] and Figure 1 shows the effect on $^{25}Mg^{2+}$ line width [$\Delta\nu_{1/2}(Mg^{2+})$] when increasing amounts of ion

(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.[4]
(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.; Nordenskiold, 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.

(10) The value of τ_c at a given temperature can be calculated from[11,12]

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

(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.

0002-7863/91/1513-675$02.50/0

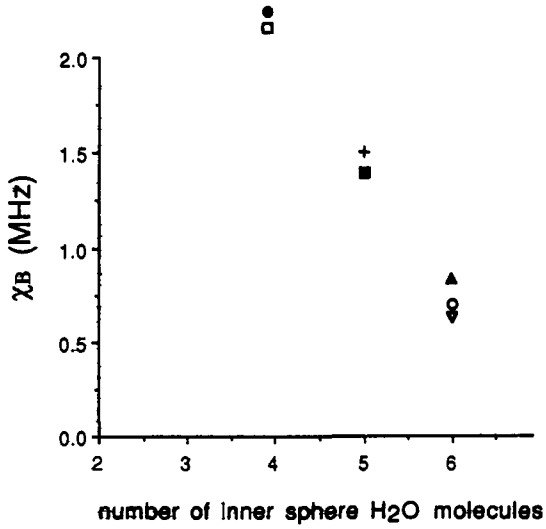

Figure 2. Variation of χ_B with number of H_2O molecules ($6-n$) in the inner coordination sphere of Mg^{2+}. Data shown corresponds to ATP^{4-} (●), ADP^{3-} (□), glucose 1-phosphate (+), glucose 6-phosphate (■), $tRNA^{Phe}$ [native (▲) and nonnative (▽) conformations], and 5S rRNA (○). Values for χ_B (±10%) were determined by line-shape analysis of data determined by systematic titration and variable-temperature experiments, and from relaxation measurements.[8,11,21]

are added to a 0.22 mM solution of *Escherichia coli* 5S rRNA. A resonance was observed after the addition of 1 equiv of magnesium, and so in contrast to most tRNAs, 5S rRNA does not possess strong Mg^{2+} binding sites (i.e., K_a are all $<10^4$ M^{-1}).[8,16] The formation of the ribosomal complex from rRNA and protein components would demand a certain flexibility on the part of the RNA and is in keeping with the absence of tight-binding Mg^{2+} domains at intrastrand cross-links, such as those found in tRNA.[16] A total line-shape analysis[17] of the data in Figure 1 for 80 bound ions[5] gave the following binding parameters: $K_a = 236 \pm 20$ M^{-1}; $\Delta G^* = (13.1 \pm 0.2) \times 10^3$ kcal; $k_{off} = 1.5 \times 10^3$ s^{-1} ($k_{on} = K_a k_{off} = 3.5 \times 10^5$ s^{-1}); $\chi_B = 0.69 \pm 0.1$ MHz. Parameter χ_B is given by e^2Qq_{zz}/h, where Q is the quadrupole moment and q_{zz} is the electric field gradient at the nucleus. The value of χ_B is dependent on the asymmetry at the metal center[18,19] and should therefore be a probe of the inner coordination sphere of the ion. The plot in Figure 2 displays a systematic variation in χ_B with substitution of the inner-sphere waters (n), reflecting the increasing electric field gradient and the greater asymmetry in the ligand environment of Mg^{2+}.[20] Previous determinations of χ_B for Mg^{2+} binding sites on proteins (troponin c, $\chi_B \sim 1.1$ MHz; calmodulin and tryptic fragments, $\chi_B \sim 1.6$ MHz; phospholipase, A_2, $\chi_B \sim 1.4$–2.3 MHz)[19,21,22] reflect a larger asymmetry and coordination number. The small value of χ_B for RNA complexes indicates retention of the hydration shell and outer-sphere coordination by $Mg(H_2O)_6^{2+}$. For free Mg^{2+} ($\tau_c \sim 0.1$–0.01 ns) with a T_1 of 173 ms, χ can be estimated from the relationship $1/T_1 = (3\pi^2/10)\chi^2(2I+3)\tau_c/[I^2(2I-1)]$ to give $\chi = 7.7$–24 kHz. The larger value for $Mg(H_2O)_6^{2+}$ bound to RNA suggests multiple hydrogen-bonding interactions between backbone phosphates, base O and N atoms, and sugar hydroxyls. Computer graphics analysis of $Mg(H_2O)_6^{2+}$ binding sites in the major and minor grooves of dsRNA suggests that five to seven H bonds can form for each hexaaquo ion.[23] This also explains the relatively large value of K_a,[24,25] which is similar to that determined for $tRNA^{Phe}$ (yeast).[8] An upper limit for the excess energy of each bound Mg^{2+} ion resulting from H-bond formation over direct phosphate coordination (which allows three to four fewer H bonds) can be estimated from the relative K_a's for magnesium binding to phosphodiesters (~ 3 M^{-1}) and RNA (~ 200–250 M^{-1}), using the relationship $\Delta G° = -RT \ln K_a$. We calculate $\Delta\Delta G° \sim 0.75$ kcal/mol per Mg^{2+} ion in favor of maximal H-bond interactions. Stabilization of the hydration sphere can therefore be ascribed to the dominance of hydrogen-bond formation.

In this paper we have demonstrated the absence of "strong" Mg^{2+} binding sites on 5S rRNA. Magnesium–RNA chemistry is likely to be dominated by hexahydrated ions held in the major and minor grooves of dsRNA by hydrogen-bonding. The coordination state of $(Mg^{2+})_{bound}$ may be deduced from χ_B. The rate of Mg^{2+} exchange ($k_{ex} \sim 1.5 \times 10^3$ s^{-1}) is rapid enough to support metalloregulatory mechanisms for conformational changes in RNA tertiary structure.[7]

Acknowledgment. We thank H. Nolier and K. Triman (U.C. Santa Cruz) for providing the overproducing *E. coli* strain for 5S rRNA and Dr. L.-Y. Hsu for invaluable assistance with graphics analysis. FT-NMR spectra (300 MHz) were obtained at The Ohio State University Chemical Instrument Center. This work was supported in part by a seed grant from the American Cancer Society, administered by The Ohio State University.

(16) Stein, A.; Crothers, D. M. *Biochemistry* **1976**, *15*, 160–68. Stein, A.; Crothers, D. M. *Biochemistry* **1976**, *15*, 157–60. Stein, M. B.; Stein, A. *Biochemistry*, **1976**, *15*, 3912. Jack, A.; Ladner, J. E.; Rhodes, D.; Brown, R. S.; Klug, A. *J. Mol. Biol.* **1977**, *111*, 315–328. Quigley, G. J.; Teeter, M. M.; Rich, A. *J. Mol. Biol.* **1978**, *75*, 64.

(17) The analysis procedure has been outlined in detail previously.[8,11,21] The similarity of $\tau_c(Mg^{2+})_{bound}$ and τ_c(RNA) precludes the influence of internal motion. Integration of resonances indicated that all of the signal was observable.

(18) Harris, R. K. *Nuclear Magnetic Resonance Spectroscopy*; Longman: Avon, 1986; pp 131–141.

(19) Drakenberg, T.; Andersson, T.; Forsen, S.; Wieloch, T. *Biochemistry* **1984**, *23*, 2387–2392.

(20) χ_B will also depend on the identity of the ligand atoms and their orientation (e.g., cis/trans geometry). Different calibration curves might be appropriate for other cases.

(21) Tsai, M.-D.; Drakenberg, T.; Thulin, E.; Forsen, S. *Biochemistry* **1987**, *26*, 3635–3643.

(22) Forsen, S.; Andersson, T.; Drakenberg, T.; Thulin, E.; Sward, M. *Fed. Proc.* **1982**, *41*, 2981–2986.

(23) Cowan, J. A.; Hsu, L.-Y., unpublished results.

(24) Typically K_a for inner-sphere complexes of monophosphate ligands are in the range 6–40 M^{-1}: Kluger, R.; Wasserstein, P.; Nakaoka, K. *J. Am. Chem. Soc.* **1975**, *97*, 4298–4303.

(25) This approach does not neglect electrostatic interactions but empirically accounts for other binding mechanisms. Polyelectrolyte theory does not consider covalent interactions.

Effect of Nonexcitonic Interactions among the Paired Molecules on the Q_y Transition of Bacteriochlorophyll Dimers. Applications to the Primary Electron Donors P-860 and P-960 in Bacterial Reaction Centers

V. Rosenbach-Belkin,† J. R. E. Fisher,‡ and A. Scherz*

Department of Biochemistry
Weizmann Institute of Science, Rehovot, Israel 76100

Received February 7, 1989
Revised Manuscript Received March 15, 1990

X-ray diffraction of reaction centers[1] (RCs) from two strains of purple bacteria, *Rhodobacter sphaeroides*[2,3] and *Rhodopseudomonas viridis*,[4] revealed that their primary electron donors, P-860 and P-960, are tight dimers of bacteriochlorophyll (Bchl)

* Recanati Career Development Chair.
† In partial fulfillment of a Ph.D. Thesis.
‡ In partial fulfillment of a M.Sc. Thesis.

(1) Okamura, M. Y.; Feher, G.; Nelson, N. In *Photosynthesis, Energy Conversion by Plants and Bacteria*; Govindjee, Ed.; Academic Press: New York, 1982; pp 221–227.

(2) Chang, C.-H.; Tiede, D.; Tang, J.; Smith, U.; Norris, J. R.; Schiffer, M. *FEBS Lett.* **1986**, *205*, 82–86.

(3) Allen, J. P.; Feher, G.; Yeates, T. O.; Komiya, H.; Rees, D. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5730–5734.

(4) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *Nature (London)* **1985**, *318*, 618–684.

0002-7863/91/1513-676$02.50/0