NMR Studies of Paramagnetic Metal Ion Interactions with Gluconate and 1,5-Gluconolactone

W. ROBERT CARPER* and DAVID B. COFFIN

Department of Chemistry, Wichita State University, Wichita, KS 67208 (U.S.A.)

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Abstract

The effect of temperature on the spin–lattice water proton relaxation rates (R_{1p}) of metal ion–gluconate and metal ion–1,5-gluconolactone interactions is determined for hexaaqua Mn(II) and Co(II) in D_2O. Activation energies are reported for manganese(II) and cobalt(II) interactions with gluconate and manganese(II) interactions with 1,5-gluconolactone. The plots of $\ln R_{1p}$ versus T^{-1} contain an inflection for Mn(II)–gluconate and Co(II)–gluconate complexes in the temperature range 20–36 °C. The results are consistent with the bent chain form of gluconate being the primary structure in solution above 35 °C.

Introduction

The spin–lattice relaxation rate ($R_1 = 1/T_1$) of water protons in hexaaqua complexes of the paramagnetic ions, Mn(II) and Co(II), is sensitive to a variety of effects [1]. One of these effects is the replacement of a water molecule with another ligand such as a sugar molecule (S) to form the species, $M(H_2O)_5(S)^{2+}$. Any structural or binding change in the sugar molecule coordinated to the metal ion may in turn be reflected by a change in its contribution to the spin–lattice relaxation time of the coordinated water molecules. While the complexity of such changes in relaxation rates may preclude their exact identification, observation of such changes are verification of molecular events occurring.

In our investigations of the enzyme gluconolactonase [2–4], it is important to detail the exact interactions between the catalytically required divalent metal ion and either the substrate, 1,5-gluconolactone, or the product, gluconate. This led to a ^{13}C NMR study of Mn(II)–gluconate interactions [5], in which we observed an unusual temperature anomaly similar to that reported in NMR studies of phase transitions [6]. This result in addition to an FT-IR study [7], suggested a conformational change in the structure of at least one of the two dominant gluconate conformations [8] and prompted this investigation.

In this paper we report the effects of temperature on the spin–lattice water proton relaxation rates ($R_{1p} = 1/T_{1p}$) of the aqua complexes of manganese(II)–gluconate, cobalt(II)–gluconate, and manganese(II)–1,5-gluconolactone complexes in D_2O. We also report the results of manganese(II)–1,5-gluconolactone water proton relaxation rate versus temperature studies.

Experimental

Gluconolactone, the sodium salt of D-gluconic acid and deuterium oxide (low paramagnetic impurity grade) were obtained from Sigma Chemical. ^1H spectra were recorded on a Varian XL-300 spectrometer operating at 300 MHz. All sugar solutions were 1 M and metal ion concentrations were 1 mM. Sample temperatures were regulated by the spectrometer and calibrated against a standard ethylene glycol solution. T_1 measurements were made using the inversion recovery method $(180°–\tau–90°–T)_n$ with $T > 5T_1$. A non-linear, three parameter fitting scheme was used to calculate T_1 values. A minimum of four spectra were obtained at each temperature and a digital resolution of 0.489 Hz was obtained for all spectra. The hydrolysis of 1,5-gluconolactone required a series of T_1 measurements as a function of time at each temperature. Separate experiments were performed at each temperature with six to eight T_1 values determined at various time intervals. These T_1 values were then plotted versus time and the resulting curve extrapolated to zero time to determine the T_1 values used in this report. Each data point represents an average of at least four measurements on separate solutions at each temperature.

The observed relaxation of water protons in a solution of a paramagnetic ion is the sum of the relaxation due to the paramagnetic ion and the

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*Author to whom correspondence should be addressed.

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relaxation in the absence of the ion. The contribution of the paramagnetic ion to the longitudinal relaxation, R_{1p}, is given by eqn. (1) derived by various workers [9–11]

$$R_{1p} = 1/p'(T_{1p} + \tau_M) = [R_{1p}(M + S) - R_{1p}(S)]/p' \quad (1)$$

where $T_{1p} \gg \tau_M$; $p' = qN_B/N_F$; M = [metal ion], S = [sugar]; N_F, N_B are nuclei in free or bound species and q = coordination number.

Results

Spin–Lattice Relaxation Rates

Table 1 contains water proton relaxation rates and correlation times (τ_c) for 1 mM solutions of $Mn(H_2O)_6^{2+}$ and $Co(H_2O)_6^{2+}$ in D_2O. The Solomon–Bloembergen equations [12, 13] describe the paramagnetic contribution to the spin–lattice (R_1) and spin–spin (R_2) relaxation rates of spin 1/2 nuclei in the coordination sphere of the paramagnetic ion. *Ab initio* calculations [14] have firmly established the validity of the point dipole approximation in the Solomon–Bloembergen equations for hydrogen atoms in a number of hexaaqua divalent metal complexes including Mn^{2+} and Co^{2+}. The Solomon–Bloembergen equation for R_{1p} is

$$R_{1p} = K[3\tau_c/(1 + \omega_I^2\tau_c^2) + 7\tau_c/(1 + \omega_s^2\tau_c^2)]/r_{MH}^6 \quad (2)$$

where $K = 2.878 \times 10^{-31}$ for Mn(II) ($S = 5/2$) and 1.234×10^{-31} for high spin ($S = 3/2$) Co(II), r_{MH} is the distance between the metal ion and the proton, ω_I and ω_s are the proton and the electron Larmor precession frequencies.

Since $\omega_s^2\tau_c^2 \gg 1$ and $\omega_I^2\tau_c^2 < 1$ for the hexaaqua complex protons [15, 16] over the temperature range studied, eqn. (2) reduces to

$$R_{1p} = 3K\tau_c/r^6 \quad (3)$$

TABLE 1. Proton relaxation rates (R_{1p}) and correlation times (τ_c) for Mn^{2+} (1 mM) and Co^{2+} (1 mM) vs. temperature

Temperature (°C)	Manganese(II)		Cobalt(II)	
	R_{1p} (s^{-1})	τ_c (ps)	R_{1p} (s^{-1})	τ_c (ps)
11	2.24	4.26	0.216	0.470
16	1.98	3.76	0.201	0.437
21	1.70	3.22	0.182	0.395
26	1.48	2.80	0.173	0.374
30	1.35	2.56	0.167	0.365
33	1.26	2.40	0.163	0.353
38	1.18	2.13	0.155	0.336
43	0.98	1.86	0.148	0.319
48	0.90	1.71	0.139	0.302
53	0.80	1.52	0.133	0.290
58	0.72	1.33	0.127	0.277

The dependence of relaxation rate with temperature indicates that the spin–lattice relaxation time, T_{1p} ($= 1/R_{1p}$) is considerably longer than τ_M, the length of time that the proton spends in the metal ion coordination sphere. This is consistent with $\omega_I^2\tau_c^2 < 1$, one of the two requirements for the reduction of eqn. (2) to (3) above. Equation (3) was used to calculate the correlation times in Table 1 where $r = 0.28$ and 0.286 nm for Co(II) and Mn(II), respectively.

The correlation times for the hexaaqua complexes of Mn(II) and Co(II) in μM metal ion solutions have been reported as 3.3 ps (27 °C) [15] and <0.5 ps (30 °C) [16] and are similar to those values listed in Table 1 obtained with 1 mM metal ion solutions. The correlation times of the hexaaqua complexes can be described by eqn. (4) over a wide range of metal ion concentrations

$$1/\tau_c = 1/\tau_e + 1/\tau_M + 1/\tau_r \quad (4)$$

where τ_e is the electron-spin relaxation time, τ_M is the lifetime(residence time) of the coordinated ligand(proton) and τ_r is the rotational relaxation time.

In summary, the fastest mechanism will determine the effective correlation time (τ_c). The electron-spin relaxation times for the hexaaqua complexes of Mn^{2+} and Co^{2+} are in the ns and ps ranges, respectively [1]. Similarly, the residence time (τ_M) for water protons in the hydration complex will exceed the reorientation time for the complex, thus eliminating this term from further consideration in eqn. (4).

The temperature dependence of τ_c, along with τ_c values in the ps range, clearly indicate that τ_c is in effect τ_r for $Mn(H_2O)_6^{2+}$ whereas both the electron-spin and rotational relaxation mechanisms will apply in the case of $Co(H_2O)_6^{2+}$. It is likely that the electron-spin relaxation mechanism dominates for $Co(H_2O)_6^{2+}$. However, it should be pointed out that the increasing domination of one mechanism occurs at the expense of the other. Finally, plots (not shown) of ln R_{1p} (Table 1) versus T^{-1} for Mn(II) and Co(II) yield activation energies of 19.2 and 8.4 kJ mol^{-1}, respectively. These may be compared to values of 23.0 and 6.1 kJ mol^{-1} for the hexaaqua complexes of Mn(II) and Co(II) obtained by Bernheim *et al.* [17] at μM metal ion concentrations.

Figure 1 contains the water proton relaxation rates, R_{1p}, for the manganese(II)–gluconate and manganese(II)–1,5-gluconolactone interactions in a ln R_{1p} versus T^{-1} plot. The data were analyzed using eqn. (1), assuming a model in which the inner coordination sphere of the metal ion contains a single sugar molecule and five water molecules. This model is consistent with previous FT-IR and NMR studies of Mg(II) and Mn(II) complexes containing gluconate or 1,5-gluconolactone [5, 7].

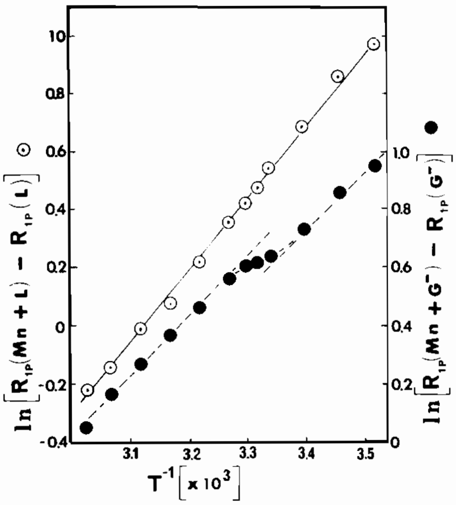

Fig. 1. Plot of ln R_{1p} for manganese(II)–1,5-gluconolactone (L) and manganese(II)–gluconate (G^-) interactions vs. temperature.

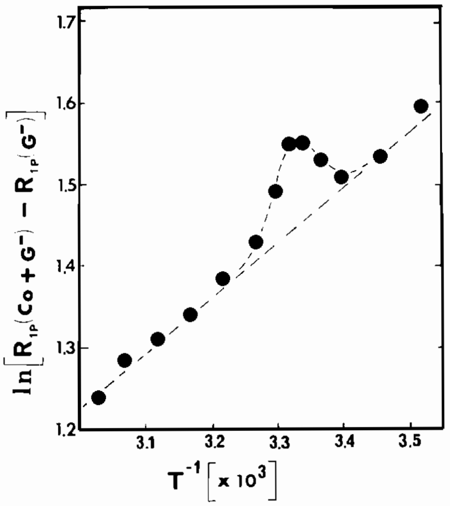

Fig. 2. Plot of ln R_{1p} for cobalt(II)–gluconate (G^-) interactions vs. temperature.

The activation energies are 20.1 and 16.3 kJ mol^{-1} for the linear portions of the manganese(II)–gluconolactone and manganese(II)–gluconate plots. There is a small but measurable inflection in the manganese(II)–gluconate R_{1p} versus T^{-1} plot, similar to those observed for conformational changes in proteins [18–20]. This inflection occurs over the same temperature range where a minimum is observed in ^{13}C NMR R_2 versus T^{-1} plots for gluconate in D_2O [1]. Addition of manganese(II) to gluconate results in loss of this minimum with the simultaneous appearance of a minimum in R_1 versus T^{-1} plots[1]. Thus, our present results parallel those observed previously in ^{13}C relaxation studies [5].

Figure 2 contains the proton relaxation rates for the cobalt(II)–gluconate interactions in a ln R_{1p} versus T^{-1} plot. As was the case for Mn(II)–gluconate, the Co(II)–gluconate proton relaxation rates are characterized by an unusual inflection over the temperature range 20–36 °C. The activation energy for the linear portion of this plot is 5.6 kJ mol^{-1} which is of the same order of magnitude as the hexaaqua complex. These results are further evidence for a minor gluconate conformational change occurring over this temperature range (20–36 °C). Unfortunately, the hydrolysis of gluconolactone is considerably enhanced in the presence of cobalt(II), thus making the measurement of R_{1p} for Co(II)–gluconolactone impossible to obtain with any reasonable degree of accuracy.

^{13}C Chemical Shift Measurements

In addition to the results contained herein and a previous ^{13}C NMR study [5] of metal–gluconate interactions, we have measured ^{13}C chemical shifts of gluconate in D_2O over the temperature range 21–53 °C. We observe only small (<0.32 ppm) upfield shifts for C2–C6 (C1 = C=O), with the largest shifts occurring for C6. C1 undergoes less than a 0.03 ppm downfield shift over the same temperature range, while C3 undergoes a slight downfield shift from 21 (72.44 ppm) to 28 °C (72.41 ppm), followed by an upfield shift to 72.66 ppm at 53 °C. These chemical shift values are far less than one would expect for major conformational changes [21, 22].

Discussion

The solid state structure of potassium gluconate contains both straight chain (A) and bent chain (B) conformations, with intra-molecular hydrogen bonds in A [23]. A NMR study of gluconic acid in DMSO indicates that the two forms in Fig. 3 exist at 30 °C [8]. The crystal structure of the manganese(II)–gluconate complex consists of two bent chain gluconate conformations which differ only in rotation about C3–C4 and C4–C5 [24]. One of these two conformations [24] is identical with form B of gluconate.

In view of the small changes in ^{13}C chemical shifts and the observed temperature anomalies in the ln R_{1p} versus temperature plots, we conclude that the two conformations (A and B) of gluconate that exist at 30 °C are replaced by a single conformation at higher temperatures. The conversion process would involve the energetic equivalent of the disruption of a single intra-molecular hydrogen bond with C3 being involved via C–C rotation and/or hydrogen

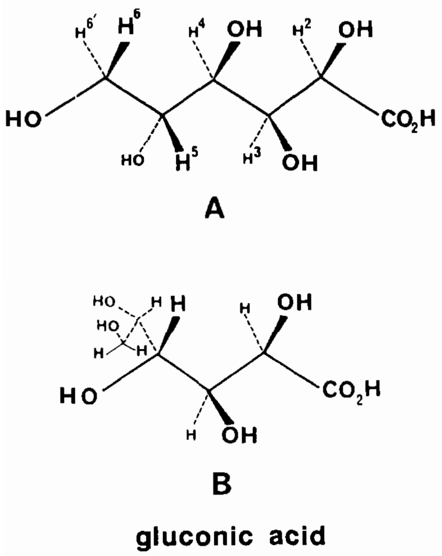

Fig. 3. Molecular structures of gluconate in solution: A, straight-chain form; B, bent-chain form.

bond breakage of formation. The conversion of the straight-chain form A to the bent-chain form B is typical of such a process, as the energetically unfavorable 1,3-parallel interactions in A are stabilized by intra-molecular hydrogen bonds [23] and requires disruption of these hydrogen bonds accompanied by rotation of the C–C axis about the C3–C4 bond.

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