Determination of the Mode of Interaction of Mn^{2+} and Cu^{2+} with *cis*-Inositol by ^{13}C NMR Spectroscopy

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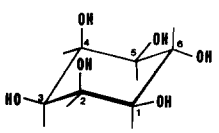
Abstract

Natural abundance ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR) was used to study the mode of binding of Mn^{2+} and Cu^{2+} to the cyclitol, *cis*-inositol. Resonance linewidths and the electron nuclear relaxation rates $[(T_1^e)^{-1}$ values] were used to establish that a unique binding site exists for these metal-ions on this cyclitol involving only the three axial hydroxyl groups. This work may aid in the development of new organometallic complexes used as paramagnetic relaxation agents in magnetic resonance imaging research.

Introduction

There has been a recent interest and focus on gaining information about the mode of binding of Gd^{3+} and Mn^{2+} to various carbohydrates [1–3], carbohydrates containing glycopeptides [4–7], and related polyols [8, 9] and cyclitols [10]. These studies were undertaken to not only elucidate the mode of interactions of the metal-ions with the various carbohydrate or carbohydrate related compounds but also to understand the nature of the spin–spin, electron–nuclear relaxation process $[(T_2^e)]$ [10]. Metal-ions such as Gd^{3+} and Mn^{2+} are of particular importance because their organic complexes may have medical applications as paramagnetic contrast agents used in nuclear magnetic resonance imaging [11–14].



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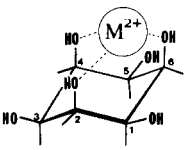

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In our previous studies dealing with the interaction of Gd^{3+} and Mn^{2+} with epi-inositol (**1**) [10] we found that a unique binding site existed for Gd^{3+} on this molecule involving the three vicinal hydroxyl groups having the axial-equatorial-axial configuration, such as the hydroxyl groups of C-2, C-3, and C-4 of **1**. This is apparently a strong metal-ion binding site for metal-ions with an ionic radius ~1.0 Å [15, 16]. Mn^{2+}, on the other hand, has an ionic radius of ~0.8 Å and was consequently too small to fit into this unique binding site on epi-inositol.

Work done by Angyal and coworkers [15, 16] indicated that *cis*-inositol (**2**), a compound similar to epi-inositol, contains three hydroxyl groups in axial positions. These groups apparently form a unique binding site for metal-ions with an ionic radius of ~0.8 Å. This binding site is too small to accomodate the larger Gd^{3+} ion [15].

In order to elucidate the nature of the *cis*-inositol metal-ion binding site and also to obtain information about the effects of the unpaired electrons spins of Mn^{2+} and Cu^{2+} on the carbon atom linewidths, we monitored the $(T_1^e)^{-1}$ values and the resonance linewidths of the *cis*-inositol carbon atoms as a function of added Mn^{2+} and Cu^{2+}.

Experimental

Synthesis of cis-inositol

1,2:3,4-Di-O-cyclohexylidene-*cis*-inositol was a gift from Dr. S. J. Angyal of the University of New South Wales, Australia. This compound was readily converted to *cis*-inositol by heating the sample in an acetic acid/H_2O solution [17] and the by-products were pumped off by successively freeze-drying the

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sample. The total conversion of 1,2:3,4-di-O-cyclohexylidene-*cis*-inositol to *cis*-inositol was monitored by ^{13}C-NMR spectroscopy which showed resonances at 74.1 and 69.3 ppm, as expected [18].

Methods

Stock solutions (0.6 M) of Mn^{2+} and Cu^{2+} were prepared by the addition of dried $MnCl_2$ and $CuCl_2$ to deionized, distilled H_2O. Additions of Mn^{2+} and Cu^{2+} to the *cis*-inositol samples were made in μl quantities, using an Eppendorf digital pipet.

^{13}C NMR spectra were recorded with a JEOL-FX90Q instrument operating at 22.5 MHz (2.1 T), as described previously [8]. ^{13}C chemical shifts are given relative to Me_4Si.

Results and Discussion

The ^{13}C NMR spectrum of *cis*-inositol gives rise to two resonances, at 74.1 ppm and 69.3 ppm. These resonances correspond to the carbon atoms (Fig. 1) which contain the axial and equatorial hydroxyl groups, respectively [18]. The fact that we do observe two resonances for the highly symmetrical molecule indicates that the chair-to-chair interconversion of the molecule is 'slow' on the NMR time scale. However, the individual structures are not totally 'frozen out' because the resonance linewidths are ~50 Hz at room temperature and can be considerably sharpened when the spectra are taken at ~2 °C [18].

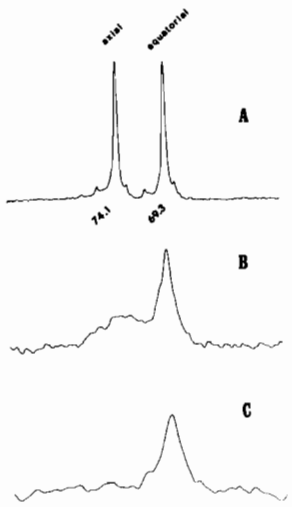

Fig. 1. The effect of Mn^{2+} on the ^{13}C resonances of the proton-decoupled, natural abundance, ^{13}C NMR spectrum of **2**. Spectra were recorded with recycle times varying from 0.3–1.5 s. The concentration of compound **2** was 390 mM in H_2O, pH ~ 7. The vertical gain of the spectra of solutions containing large portions of paramagnetic relaxation-reagent was increased slightly, so that broadening effects could be clearly observed. (A) Sample contained no Mn^{2+} and required 9500 accumulations. A line-broadening factor of 1.0 Hz was used during the data processing. (B) Sample contained 2.1 mM Mn^{2+}, and required 18,351 accumulations. A line-broadening factor of 8.1 Hz was used during the data processing. (C) Sample contained 10 mM Mn^{2+} and required 3500 accumulations. A line-broadening factor of 15.0 Hz was applied during the data processing.

In view of the problems mentioned above concerning the resonance linewidths, we decided to pursue our investigation of the binding of Mn^{2+} and Cu^{2+} to cis-inositol at room temperature but we added $ZnCl_2$ (to make a 0.4 M solution) in an attempt to 'freeze out' the various structures of *cis*-inositol; the addition of $ZnCl_2$ did reduce the resonance linewidths to ~25 Hz. Besides allowing us to reduce the linewidths of the *cis*-inositol ^{13}C resonances, the addition of $ZnCl_2$ to our sample served another purpose. Since Zn^{2+} is diamagnetic and has approximately the same ionic radius as Mn^{2+} and Cu^{2+}, it serves as a diamagnetic probe for the structural changes that are to be expected upon chelation of Mn^{2+} and Cu^{2+} to *cis*-inositol, but which may not be observable because of their paramagnetic properties.

Figure 1 shows the effects of added Mn^{2+} on the spectra of *cis*-inositol. Clearly the resonance of the carbon atoms containing the axial hydroxyl groups broadens immediately while the resonance of the carbon atoms containing the equatorial hydroxyl groups appear less affected. Note how little Mn^{2+} is required relative to *cis*-inositol in order to observe a significant effect (see Fig. 1B). In qualitative terms, we can say that Mn^{2+} binds specifically to the axial hydroxyl groups of *cis*-inositol.

Figure 2 shows the effects of added Cu^{2+} on the spectra of *cis*-inositol. The effects are similar to those shown in Fig. 1 dealing with Mn^{2+} and we can therefore conclude that Cu^{2+} also binds in a unique site involving the three axial hydroxyl groups. Note that we have not broadened the resonances of the carbon atoms containing the axial hydroxyl groups completely, even though 17.6 mM Cu^{2+} has been introduced. This is due to the fact that Mn^{2+} has a spin of $\frac{5}{2}$ and Cu^{2+} only a spin of $\frac{1}{2}$. If the T_2^e were strictly dominated by a dipolar electron-nuclear relaxation mechanism then one could conclude that the difference in the effects on the linewidths should be ~11.7 [determined from S(S + 1)]. Since Cu^{2+} is not a 'pure' relaxation reagent but also a shift reagent, the addition of Cu^{2+} (to produce a 0.5 M solution) (Fig. 2C) shifted the resonances somewhat. Hence, Fig. 2C was contracted by a factor of 5 in order to bring the resonances in ling with those observed in Figs. 2A and 2B. Although this does distort our absolute linewidths of these resonances, it none the less does give us a clear picture of the relative linewidths.

In order to gain further structural information about the binding of Mn^{2+} and Cu^{2+} to *cis*-inositol,

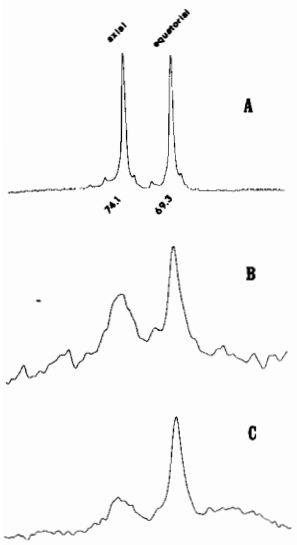

Fig. 2. The effect of Cu^{2+} on the ^{13}C resonances of the proton-decoupled, natural abundance, ^{13}C NMR spectrum of 2. Spectra were recorded with recycle times varying from 0.1–1.5 s. The concentration of compound 2 was 390 mM in H_2O, pH ~ 7. The vertical gain of the spectra of solutions containing large portions of paramagnetic relaxation-reagent was increased slightly, so that broadening effects could be clearly observed. (A) Same as 1A. (B) Sample contained 17.6 mM Cu^{2+}, and required 5000 accumulations. A line-broadening factor of 15 Hz was used during the data processing. (C) Sample contained 500 mM Cu^{2+} and required 168299 accumulations. A line-broadening factor of 15 Hz was applied during the data processing.

we also measured the electron nuclear spin-lattice relaxation rates [$(T_1^e)^{-1}$ values] of the various carbon atoms as a function of added Mn^{2+} and Cu^{2+}. Since the T_1^e is dominated by a dipolar interaction between the unpaired electron(s) and the carbon nucleus, a direct distance dependence (r^{-6}) can be obtained. This is not necessarily true for the T_2^e, which sometimes makes its use limited unless it can be proven that T_2^e is dominated by a dipolar mechanism. Strangely we could find no significant difference between the $(T_1^e)^{-1}$ values of the axial or equatorial carbon atoms; plots of $(T_1^e)^{-1}$ *vs.* added Mn^{2+} and Cu^{2+} gave slopes of 5.55×10^3 s^{-1} $[M]^{-1}$ and 0.763×10^3 s^{-1} $[M]^{-1}$, respectively. The ratio of these slopes was found to be 7.5 which is similar to the theoretical value of 11.7 for the effects of Mn^{2+} *vs.* Cu^{2+}. The slight difference may arise from the fact that Cu^{2+} and Mn^{2+} may not bind in exactly the same manner to *cis*-inositol.

Since we observe almost identical slopes for the carbon atoms containing the axial and equatorial hydroxyl groups in our plots of $(T_1^e)^{-1}$ *vs.* $[Cu^{2+}]$ and $[Mn^{2+}]$, this must indicate that these carbon atoms are equidistant from the metal-ions. This can be only rationalized if the chair conformation of the ring system has become somewhat distorted in driving the system to a near planar form. The resonance linewidths would not indicate this if the $(T_2^e)^{-1}$ values are dominated by a scalar mechanism (through bond transmission of the unpaired electron spin density).

Thus the only way to rationalize our data is to invoke a slightly distorted chair structure for the binding of Mn^{2+} and Cu^{2+} to *cis*-inositol. This distorted structure may have gone unnoticed in our spectra (via chemical shifts and linewidths) because our linewidths were already 30–50 Hz and therefore a change may not even be observable in our room temperature spectra with the addition of Zn^{2+}.

In order to investigate this point we decided to run a few ^{13}C NMR spectra of *cis*-inositol with and without metal at ~2 °C. At this temperature we found the resonance line widths to be 7 Hz. Upon addition of Zn^{2+}, the resonances broadened to 15 Hz and the carbon atoms containing the axial and equatorial hydroxyl groups shifted 0.5 and 0.2 ppm, respectively.

In order to investigate whether $(T_2^e)^{-1}$ values are dominated by a scalar or dipolar mechanism, we calculated various (T_1^e):(T_2^e) ratios. This ratio is known to show whether a scalar or dipolar mechanism dominates the T_2^e relaxation [10, 19, 20]. A value of 1.17 should be obtained if the T_2^e relaxation process is totally dominated by a dipolar mechanism [10, 19, 20]. A value of 1.75 is obtained if a scalar mechanism contributes 50% to T_2^e. In the case of Mn^{2+} we obtained values of 5.8 and 1.4 for the T_1^e/T_2^e ratios for the carbon atoms containing the axial and the equatorial hydroxyl groups, respectively. In the case of Cu^{2+}, we obtained values of 2.4 and 1.7 for the T_1^e:T_2^e ratios observed for the carbon atoms containing the axial and the equatorial hydroxyl groups, respectively. Clearly, these results indicate that T_2^e relaxation for the carbon atoms containing the axial hydroxyl groups is dominated by a scalar mechanism.

A number of conclusions can be drawn from our work:

(i) *cis*-Inositol apparently contains a unique binding site for Mn^{2+} and Cu^{2+} which involves the three axial hydroxyl groups.

(ii) The binding of these metal-ions to *cis*-inositol may distort the chair conformation slightly.

(iii) The resonance line broadening experiments and T_1^e analysis clearly indicate that the predominant mechanism in the T_2^e relaxation process is a scalar mechanism. Nevertheless, in an aliphatic polyol system like ours, qualitative metal-ion binding information can still be gained from monitoring the line-broadening as a function of added Mn^{2+} and Cu^{2+}.

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