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Note

Use of electron-nuclear relaxation-rates to determine Mn^{2+}–methyl D-galactopyranoside interactions

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We have previously employed the selective, line-broadening technique in order to gain information about the mode of interaction of such metal ions as Gd^{3+} and Mn^{2+} with *N*-acetyl-α-D-neuraminic acid[1] and the carbohydrate residues of oligosaccharides[2], glycoproteins[2], and glycopeptides[3–5]. Moreover, we have also utilized this technique in order to investigate the stereochemistry of the interactions of Gd^{3+} and Mn^{2+} with several synthetic D-gluconamides, which may be precursors for new, metal-ion chelating agents[6].

The use of certain metal-ions, especially Mn^{2+}, as selective, line-broadening agents for obtaining quantitative, distance information about specific, metal-ion–ligand structures has recently come into question, because the electron nuclear T_2^e (spin–spin) relaxation was, in some cases, shown to be dominated by a scalar relaxation mechanism. This would pose severe limitations on the use of the selective, line-broadening technique for gaining metal-ion–ligand, carbon-distance information, because the scalar contribution to T_2^e does not contain a simple, distance dependence[7,8]. In order to gain a more concise picture of the metal-ion–ligand interaction, electron-nuclear T_1^e (spin–lattice) relaxation of ligand carbon atoms are measured, because the T_1^e relaxation is dominated by dipolar interaction between the carbon nucleus and the unpaired electrons of the metal ion, and this interaction contains an r^{-6} distance-dependence[8].

It is because of the possible discrepancies that may exist in studying metal-ion–carbohydrate interactions by the two methods in question that we present herein the use of electron-nuclear relaxation rates $[(T_1^e)^{-1}]$ to determine the interactions of Mn^{2+} with methyl α-D-galactopyranoside (**1**) and methyl-β-D-galactopyranoside (**2**). These model compounds were chosen for the present studies because the selective, line-broadening technique had been employed in earlier studies dealing with the interactions of Mn^{2+} with glycopeptides containing α- and β-D-

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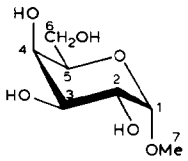

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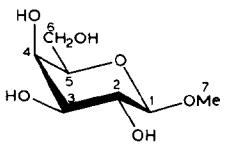

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galactopyranose[3–5]. The results of the Mn^{2+} interaction with the model compounds from this study (monitoring ^{13}C, T_1^e values) are similar to our previous studies dealing with the interactions of Mn^{2+} with α- and β-D-galactopyranose residues of various glycopeptides that used the selective line-broadening technique.

EXPERIMENTAL

Methyl α-D-galactopyranoside (**1**) and methyl β-D-galactopyranoside (**2**) were purchased from Sigma Chemical Co., St. Louis, MO. A stock solution of Mn^{2+} was prepared as previously described[1]. Additions of the Mn^{2+} stock solution to the n.m.r. samples were made in μL quantities, using an Eppendorf digital pipet.

^{13}C-N.m.r. spectra were recorded with a JEOL-FX90Q instrument, operated in the F.t. mode by the use of quadrature detection as described earlier[1]. Measurements of T_1 were made by using the partially relaxed, Fourier transform (p.r.F.t.) method (180–τ–90), with eleven τ values. Values of T_1 were calculated by using the linear, least-squares program provided by JEOL. Values of $(T_1^e)^{-1}$ were calculated from the relationship $(T_1^e)^{-1} = (T_1^{-1})_{Mn^{2+}} - (T_1^{-1})_0$, where $(T_1^{-1})_{Mn^{2+}}$ and $(T_1^{-1})_0$ are the inverse, spin–lattice relaxation-times, with and without added Mn^{2+}, respectively.

RESULTS AND DISCUSSION

Figs. 1 and 2 show the linear, least-squares fit of our data for the effects of added Mn^{2+} on the nuclear-relaxation rates $[(T_1^e)^{-1}]$ of the various carbon atoms of ~M solutions of **1** and **2**. Assignments of the resonances in our spectra to specific carbon atoms were based on our published data for various D-galactopyranosides[3–5]. No corrections were made for possible outer-sphere relaxation contributions to the ^{13}C, T_1^e values[8].

In Figs. 1 and 2, the steepness of the slope of the line indicates the strongest Mn^{2+} interactions with the respective carbohydrate oxygen atoms. The concentration of Mn^{2+} needed (relative to M solutions of model compounds) to permit observation of the effects is such that the Mn^{2+}–D-galactopyranoside interactions must be considered weak[8], and an outer-sphere process may contribute somewhat to the relaxation of the carbon nuclei. Fig. 1 shows that there are no great differences in the slopes pertaining to the effects of added Mn^{2+} on the $(T_1^e)^{-1}$ values for the various carbon atoms. Although it would appear that the oxygen atoms on C-6, C-3,

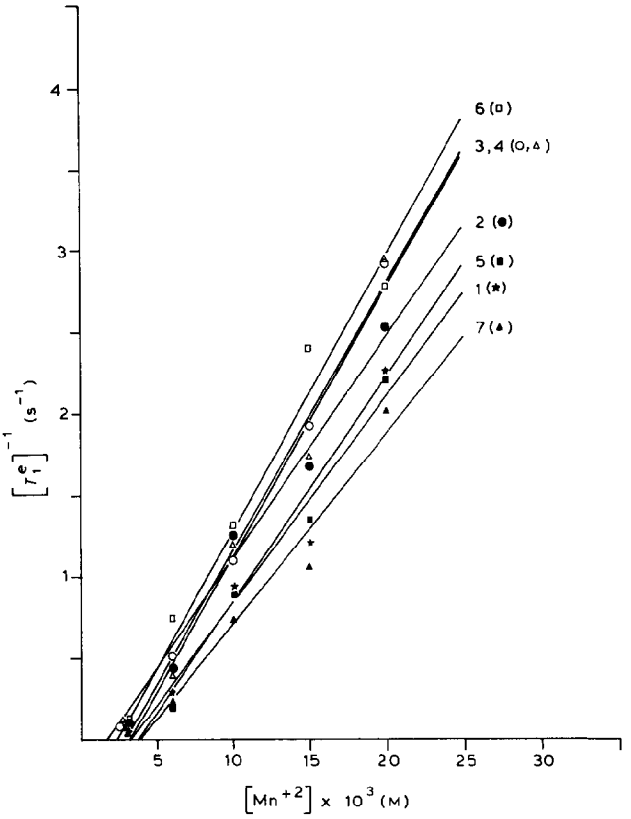

Fig. 1. The effects of added Mn^{2+} on the ^{13}C, T_1^e values of compound **1**. [The concentration of **1** was M (in H_2O), at pH ~7.0. The numbers on the graph refer to specific carbon atoms of **1**.]

and C-4 may be weak binding-sites for Mn^{2+}, the carbon atoms that do not bear hydroxyl groups (C-7, C-1, and C-5) appear to be the least affected.

Fig. 2 shows a picture somewhat similar to that observed in Fig. 1. The ^{13}C, electron-nuclear relaxation-rates of carbon atoms bearing no hydroxyl group (C-1, C-5, and C-7) appear to be the least affected by the increased additions of Mn^{2+}. The electron-nuclear relaxation-rates of C-2 and C-3 may be somewhat affected by added Mn^{2+}, and that of C-6 seems to be noticeably affected. This indicates that a "relatively" strong, interaction site exists near C-6 (probably O-6), and weaker interaction sites are present near C-2 and C-3, involving their respective oxygen atoms.

The results obtained from this work are similar to those obtained for metal ion–D-galactopyranose by using D-galactosylated peptides. The minor differences that do exist probably result from the fact that amino acids may mediate Mn^{2+} binding in the vicinity of C-1. The use of electron-nuclear relaxation-rates to determine Mn^{2+} binding to various polyols may be put to better use if a polyol is chosen that has a more appropriate, metal-ion-binding geometry[9].

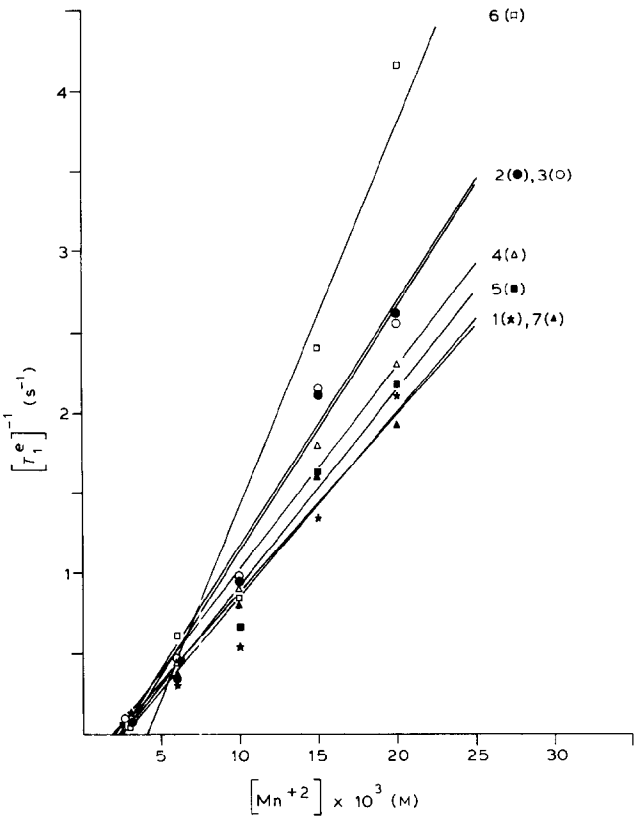

Fig. 2. The effects of added Mn^{2+} on the ^{13}C, T_1^e values of compound **2**. [The concentration of **2** was M (in H_2O), at pH ~7.0. The numbers on the graph refer to specific carbon atoms of **2**.]

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