Cr^{3+}–*cis*-Inositol: a Possible Precursor for New Contrasting Agents Used in Magnetic Resonance Imaging

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Because of the recent interest in magnetic resonance imaging, a need has arisen for certain metal-ion chelates that act as contrasting agents for enhancing images. Typically, Gd^{3+} and Mn^{2+} may be used as metal-ions in these complexes because they contain a large number of unpaired electrons; the chelating agent may be EDTA, DPTA, or even oxalates [1–6]. The contrasting agent must be chosen with great care because dissociation of the metal ion complex can provide soluble metal-ions and chelates which may be poisonous or debilitating in nature. Therefore, it is necessary to search for a metal-ion–organic complex that does not readily dissociate and which will also not be detected as a foreign substance within the body.

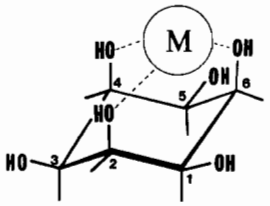
In this report we show that Cr^{3+} complexes to *cis*-inositol, a naturally occurring compound related to myo-inositol ('meat sugar'), may be a precursor for new contrasting agents that can be developed using inositols as starting materials.

Materials and Methods

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 converted to *cis*-inositol as previously described [7]. Metal-ions were added to the *cis*-inositol in μl quantities using an Eppendorfer digital pipet. ^{13}C NMR spectra were recorded with a JEOL-FX90Q instrument operating at 22.5 MHz (2.1 T) using a 10 mm sample tube, as previously described [8]. ^{13}C chemical shifts are given relative to Me_4Si. In the experiments involving Cr^{3+}–*cis*-inositol, a quantitative amount of acetone was added to our concentric 5 mm locking tube so that the peak intensity of the non-complexed *cis*-inositol could be monitored quantitatively.

Results and Discussion

Scheme 1 shows the configuration of *cis*-inositol and the manner in which some metal-ions may bind



Scheme 1.

TABLE 1. Slopes of the Plots of ^{13}C-$(T_1^e)^{-1}$ *vs.* Metal-ion Concentration for *cis*-Inositol

Metal-ion	Observed slope[a]	
	C-1,3,5	C-2,4,6
Mn^{2+} [b]	5.55	5.55
Cu^{2+} [b]	0.76	0.76
Cr^{3+}	0.20[c]	0.20[c]

[a]In 10^3 s^{-1} M^{-1}. [b]See ref. 7. [c]For residual non-chelate *cis*-inositol. Because the resonance of limited non-chelated *cis*-inositol was very small at high Cr^{3+} concentrations, scattered slopes were obtained. Resonances also broadened at higher Cr^{3+} concentrations due to outer sphere interactions. Sample contains 300 mmol Zn^{2+}.

to this compound [7]. The three axial hydroxyl groups provide the metal-ion binding site for metals of ionic radii ~0.8 Å [9, 10]. Larger metal-ions, such as Gd^{3+}, may be chelated to *cis*-inositol via the oxygen atoms attached to C-2, C-3, and C-4 [11].

The proton-decoupled, natural abundance ^{13}C NMR spectrum of *cis*-inositol gives rise to two signals at 74.1 and 69.3 ppm, which have been assigned to the carbon atoms containing the axial and equatorial hydroxyl groups, respectively. At ambient temperatures the linewidths of the resonances are ~30 Hz, whereas, at 4 °C they are ~4 Hz. The resonances coalesce at temperature greater than 55 °C, indicating that the chair to chair interconversion of this symmetrical molecule has become fast on the NMR time scale.

We have previously shown that the binding process of Mn^{2+} and Cu^{2+} to *cis*-inositol is in fast exchange limit on the NMR time scale [7]; only spectra averaged of the bound and unbound states are observed. It was shown that the line broadening of equatorial and especially axial carbon resonances was dominated by a scalar interaction between the unpaired electrons of Mn^{2+} and Cu^{2+} and the carbon nuclei. The slopes of the plots for $(T_1^e)^{-1}$ *vs.* metal-ion concentrations are provided in Table I. In order to explain the fact that the electron-nuclear relaxation rates for the equatorial and axial carbons were the same we invoked the fact that the structure of the ring was pushed towards a more planar form in order to

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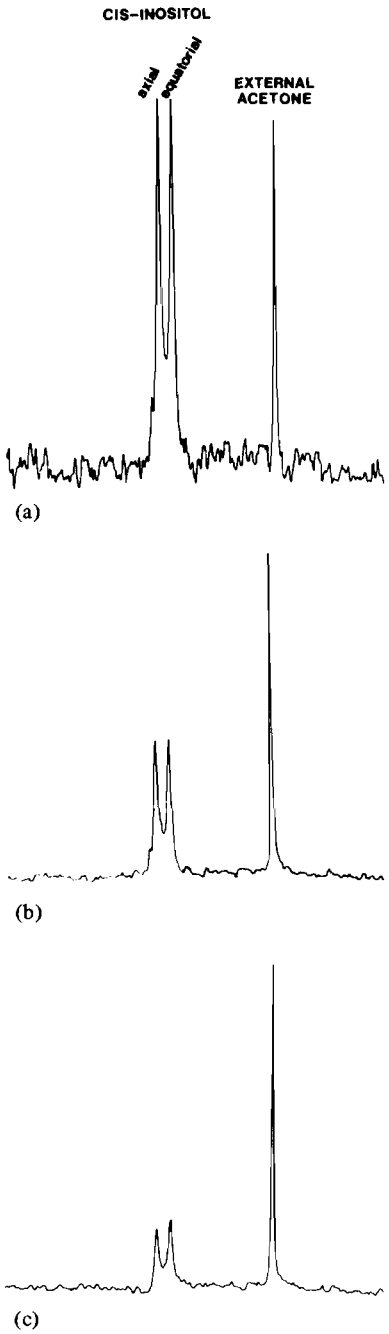

Fig. 1. The effect of Cr^{3+} on the ^{13}C resonances of the proton-decoupled, natural abundance, ^{13}C NMR spectrum of *cis*-inositol. Spectra were all recorded with a recycle time of 8.25 s and a line-broadening factor of 15.0 Hz was applied during the data processing. The concentrically inserted 5 mm locking tube contained D_2O/acetone. The external acetone peak was used as an external intensity standard. The concentration of *cis*-inositol was 390 mmol in H_2O (pH ~7.0). (a) Sample contained no Cr^{3+} and required 1178 accumulations. (b) Sample contained 1.5 mmol Cr^{3+} and required 4000 accumulations. (c) Sample contained 15 mmol Cr^{3+} and required 3762 accumulations.

accommodate these metal-ions. This fact may have since been corroborated by Angyal and coworkers [11].

Because of the ongoing fast exchange in the binding of *cis*-inositol and Mn^{2+} and Cu^{2+}, these complexes do not make good candidates for contrasting agents. Cr^{3+} on the other hand, has a smaller ionic radius (0.63 Å) and may interact with the triaxial oxygen atoms without perturbing the structure of the molecule. Moreover, recent evidence by Angyal [12] indicates that a tripositive cation forms stronger complexes than dispositive cations do with muellitol, a 1,3,5 triaxial hydroxyl containing compound.

Addition of Cr^{3+} to *cis*-inositol solution (with and without 300 mmol Zn^{2+} present) resulted in the gradual and equal decrease in the intensity of both *cis*-inositol carbon resonances; this was quantitatively measured using a 10% acetone/D_2O in a concentrically inserted 5 mm locking tube; see Fig. 1. The fact that the resonances did not broaden appreciably (except at high Cr^{3+} concentrations) and equally decreased in intensity indicates that under these conditions the interaction of Cr^{3+} with *cis*-inositol was 'slow' on the NMR time scale. An attempt to nudge this interactions into the 'fast' exchange region by raising the temperature would only have coalesced the resonances and also would not have resulted in any additional information. The $(T_1^e)^{-1}$ for the residual resonances after the addition of Cr^{3+} is shown in Table I. Clearly this must result from outer-sphere electron-nuclear relaxation.

A plot of our ^{13}C *cis*-inositol data *versus* the amount of Cr^{3+} added should provide information about the stoichiometry of this complex. Our data clearly showed that Cr^{3+} would appear to chelate with at least 4 *cis*-inositol molecules. A better value could not be obtained because the resonances eventually exhibited line-broadening at higher Cr^{3+} concentrations. This result would tend to indicate that if Cr^{3+} chelates to one *cis*-inositol molecule via the three triaxial oxygen atoms, it must bind to three equatorial oxygens of other *cis* inositol molecules. Or another way to visualize this would be to invoke that Cr^{3+} is chelated of six equatorial oxygen atoms by six different *cis*-inositol molecules, although this may involve steric crowding. A technique such as X-ray diffraction may be required for further insight into the nature of the complex.

If Cr^{3+} is indeed chelated by a *cis*-inositol molecule in the triaxial oxygen pocket then our results indicated that Cr^{3+} complex of the naturally occurring compound *cis*-inositol may be a good starting point for the development of contrasting agents. There are several possibilities in modifying *cis*-inositol that would provide for a better contrasting agent. One is to form a dimer with ether bridges connected via the axial oxygens. This would allow

the Cr^{3+} to be encapsulated. Another method would be to put a 'cap' on the open portion of the molecule, starting with a compound such as muellitol.

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