Carbohydrate Research, 187 (1989) C6–C8
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

Carbohydrate contrast agents for nuclear magnetic resonance imaging: enhancement of the relaxation of water by paramagnetic metal complexes of polysaccharides

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(Received Sepember 28th, 1988; accepted for publication, January 10th, 1989)

It is well known that paramagnetic metal ions enhance the spin–lattice relaxation rates of water. The extent of the enhancement increases with the number of unpaired electrons; hence, cupric, ferrous, ferric, manganous, and gadolinium ions have pronounced effects, whereas diamagnetic ions, such as calcium, are essentially inert[1,2]. More subtly, the enhancement depends on the rate of molecular motion of the paramagnetic centre, slower tumbling causing a greater enhancement of relaxation (assuming that all other variables are held constant). More obviously, the overall effect on the water will increase with the rate at which water molecules diffuse through the sphere of influence of the paramagnetic centre.

It is the last two considerations which prompted us to consider the role of paramagnetic complexes of polysaccharides. Such derivatives provide a highly hydrophilic environment for the sequestered metal ions, especially when the spacer arm connecting the paramagnetic centre to the polysaccharide backbone is itself hydrophilic. Furthermore, by appropriate selection of the saccharide, it is possible to vary the overall rate of molecular motion over a wide dynamic range.

The present work compares metal complexes of (*a*) a typical polysaccharide, Chelating Sepharose 6B, in which iminodiacetic acid moities are coupled by hydrophilic spacer arms to epoxy-activated Sepharose 6B (**1**; Pharmacia Ltd.; mesh size 45–160 μm; exclusion limit 10,000–5,500,000 daltons); and (*b*) a typical hydrophobic polymer, Chelex-100, in which the same iminodiacetic acid ligands are attached to a polystyrene lattice (**2**; Bio-Rad Laboratories Ltd.; mesh size 200–400 μm; effective pore size, large). Samples of each material were immersed in 0.1M solutions of the appropriate metal salts for 2 days, then thoroughly rinsed with distilled water (to remove excess of metal ions), vacuum-dried, and stored in a

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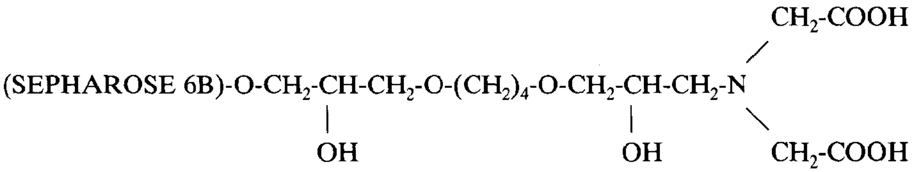
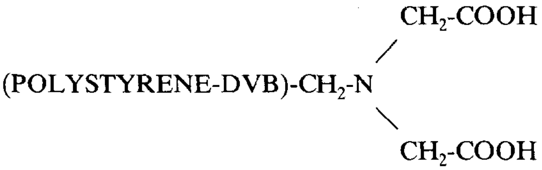
0008-6215/89/$ 03.50

desiccator. Complexes containing sodium(I), zinc(II), lanthanum(III), calcium(II), iron(II), copper(II), manganese(II), iron(III), and gadolinium(III) ions were prepared and the extent of metallation was determined by atomic absorption spectroscopy.



$$\text{(SEPHAROSE 6B)-O-CH}_2\text{-}\underset{\displaystyle\text{OH}}{\text{CH}}\text{-CH}_2\text{-O-(CH}_2)_4\text{-O-CH}_2\text{-}\underset{\displaystyle\text{OH}}{\text{CH}}\text{-CH}_2\text{-N}\begin{matrix}\diagup\ \text{CH}_2\text{-COOH}\\ \diagdown\ \text{CH}_2\text{-COOH}\end{matrix}$$

1



$$\text{(POLYSTYRENE-DVB)-CH}_2\text{-N}\begin{matrix}\diagup\ \text{CH}_2\text{-COOH}\\ \diagdown\ \text{CH}_2\text{-COOH}\end{matrix}$$

2

All n.m.r. relaxation measurements were made on 24% w/w suspensions of the fully metallated material in 2% carboxymethylcellulose gel. These were contained in either 5-mm diameter n.m.r. tubes, when using a Varian VXR-300 spectrometer (26°), or 15-mm diameter glass vials, when using an imaging spectrometer based on an Oxford Instruments 31-cm, horizontal bore magnet coupled to an Oxford Research Systems Biospec II console operating at ~85 MHz (21°). The inversion recovery sequence[1,3] with phase cycling and a composite 180° pulse[4-6] was used, and the spin–lattice relaxation rates (R_1-values) were calculated from an exponential fit of the data. The errors associated with these values were found to be between 1% and 10%.

At both operating frequencies, the normal R_1-value for water was found to be 0.35 s^{-1}; 2% carboxymethylcellulose increased this to 0.46 s^{-1}. Addition of 24% w/w of the dried, non-metallated resin **1** gave an R_1-value of 5 s^{-1}. However, addition of 24% w/w of the dried iron(III) complex of **1** [2.5% (1mM Fe^{3+}] gave an R_1-value of 400 s^{-1}, whereas a similar suspension of the dried calcium complex of **1** gave an R_1-value of 1.6 s^{-1}, thereby confirming previous results that diamagnetic ions have relatively little relaxation enhancement. Thus, **1** containing 2.5% (1mM) Fe^{3+} enhanced the relaxation rate of water 1150-fold. This effect far exceeds the 30-fold enhancement produced by a 1mM suspension of iron(III) chloride in 2% carboxymethylcellulose and thereby provides good evidence for the importance of molecular tumbling on relaxation enhancement.

Measurements made under identical conditions using **2** showed parallel

TABLE I

VARIATION IN THE SPIN-LATTICE RELAXATION RATES OF RESIN SUSPENSIONS WITH THE EXTENT OF METALLATION

Extent of metallation of resin **1**	*R_1 of 24% w/w suspension of resin* **1** *(s^{-1})*	*Extent of metallation of resin* **2**	*R_1 of 24% w/w suspension of resin* **2** *(s^{-1})*
1.6% Fe(II)	25.0	4.7% Fe(II)	1.2
2.5% Cu(II)	27.4	6.3% Cu(II)	7.1
1.3% Mn(II)	61.4	5.1% Fe(III)	23.3
2.5% Fe(III)	400.0	4.4% Mn(II)	27.0
3.1% Gd(III)	800.0	4.8% Gd(III)	41.7

behaviour. Importantly though, the iron(III) complex containing 5.1% (9mM) Fe^{3+} gave an R_1-value of 23.5 s^{-1}, which corresponds to a 70-fold enhancement in the relaxation rate of water. Ignoring the fact that any difference in molecular motion between **1** and **2** would favour the relaxivity of the latter, this comparison gives a clear indication of the substantial advantage of having a hydrophilic environment; for equal quantities of Fe^{3+}, **1** is ~35-fold more efficient a relaxation agent than **2**. Similar trends were observed for the relaxivities of the other paramagnetic complexes of **1** and **2** studied (Table I).

The use of these materials as contrast media in magnetic resonance imaging (m.r.i.) depends on the fact that the intensity displayed in an image is dependent upon the relationship between the rates of intrinsic relaxation (both R_1 and the spin–spin lattice relaxation rate, R_2) of the samples and the time constants of the measurements involved.

To the best of our knowledge, this is the first reported use of metal complexes of a polysaccharide as m.r.i. contrast agents. Given the diversity of polysaccharides to which metal chelating ligands can be attached, it is clear that there is ample scope for subtle molecular tailoring.

ACKNOWLEDGMENTS

We thank Dr. Herchel Smith for his financial support of this work through an Endowment (to L.D.H.) and a research fellowship (to J.H.B.), and also the S.E.R.C. for funds for the 300-MHz n.m.r. instrument.

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