Metal coordination to carbohydrates. Structures and function

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CONTENTS

ABBREVIATIONS

AnManOH 2,5-anhydro-D-mannitol

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0010-8545/93/$24.00

Ara	L-arabinose
Fru	D-fructose
Gal	D-galactose
$GalNH_2$	2-amino-2-deoxy-D-galactose
GalNAc	2-acetamido-2-deoxy-D-galactose
GalpA	D-galactopyranosiduronic acid
Glc	D-glucose
GlcA	D-gluconic acid
$GlcNH_2$	2-amino-2-deoxy-D-glucose
GlcNAc	2-acetamido-2-deoxy-D-glucose
GlcNS	2-deoxy-2-sulfamido-D-glucose
GlupA	D-glucopyranosiduronic acid
GulpA	L-gulopyranosiduronic acid
IdopA	L-idopyranosiduronic acid
Lzx	D-lyxose
Man	D-mannose
$ManNH_2$	2-amino-2-deoxy-D-mannose
ManpA	D-mannopyranosiduronic acid
NeuNAc	5-acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid
NeuGc	5-glycoylamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid
Rib	D-ribose
Xyl	D-xylose
ATP	adenosine-tri-phosphate
CD	circular dichroism
DNA	deoxyribonucleic acids
DP	degree of polymerization
ESR	electron spin resonance
IR	infrared spectroscopy
NMR	nuclear magnetic resonance
NOE	nuclear overhauser enhancements
RNA	ribonucleic acids
T_1	NMR spin-lattice relaxation time
T_2	NMR spin-spin relaxation time
TLC	thin layer chromotography
UV-Vis	ultraviolet-visible spectroscopy
$^4C_1$	chair conformation of pyran ring with C4 above and C1 below the plane of the ring
^2S_O	Twist-boat conformation of pyran ring with C2 above and the ring O below the plane of the ring
$^1C_4$	chair conformation of pyran ring with C1 above and C4 below the plane of the ring

In the figures displaying the reducing sugars, the exact form and anomericity is indicated but all other tautomers are possible depending on the experimental conditions. For example, hexoses can exist in cyclic pyranose and furanose forms besides the open chain forms. As well the anomeric carbon can exist in two epimers designated α and β. It should be noted that the D-hexoses usually occur in the $^4C_1$ pyranose conformation whereas the L-hexoses occur in the opposite $^1C_4$ pyranose chair. The abbreviations do not specifically indicate a particular species. In the case of glycosides mutarotation is blocked and the conformation shown in the figures corresponds to the predominant form. Some representative monosaccharides are shown in Fig. 1.

For metal ions, if the oxidation state is known then it is specified, otherwise the atomic symbols without a specific oxidation state are used as generic terms.

A. INTRODUCTION

Carbohydrates are the most abundant class of compounds by weight in the biosphere [1]. Since they also have an abundance of electronegative functional groups, it is not surprising that they interact with metal cations. Furthermore, the

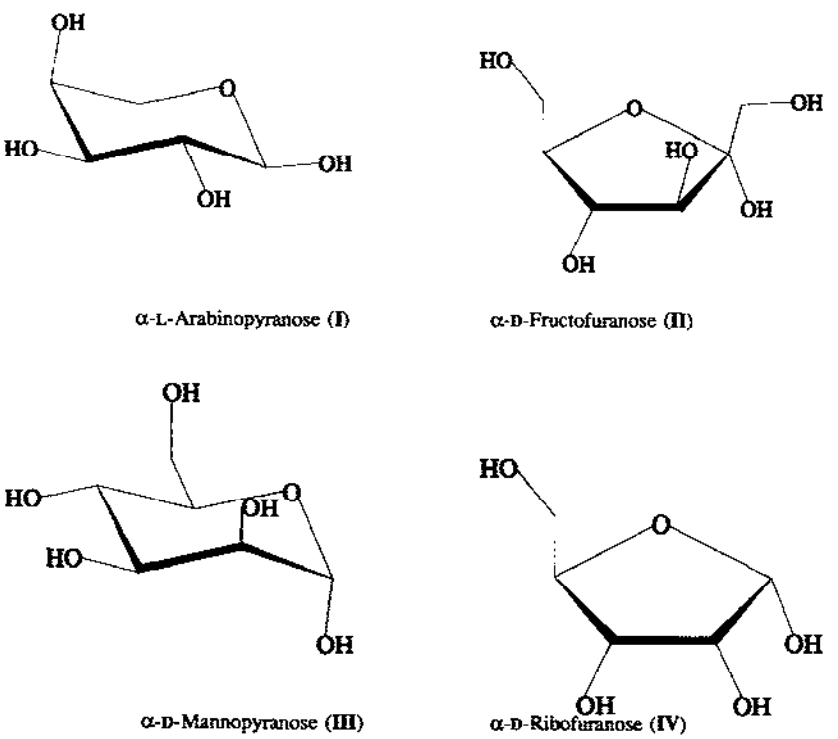


Fig. 1. Chemical structures of monosaccharides α-L-arabinopyranose (I), α-D-fructofuranose (II), α-D-mannopyranose (III) and α-D-ribofuranose (IV).

existence of a large number of monosaccharides with only small differences in size, stereochemistry and functional groups and the almost infinite possibilities when these monosaccharides are linked together to form oligosaccharides and polysaccharides creates a wealth of potential ligands which are only just beginning to be investigated. Most of the well-documented cases involve macromolecular polysaccharides, which participate in the regulation of the flow of metal ions across cell walls [2]. Such systems appear to operate in all organisms. By way of examples, we will discuss molybdenum transport in bacteria [3], iron uptake in plants [4] and nickel metabolism in human kidneys [5].

The biological role of metal carbohydrate interactions has been studied from a variety of perspectives. Thus, microbiologists, physiologists and botanists endeavour to understand both essential and toxic metal metabolism. Similarly, biochemists and chemists aim to identify the compounds involved and to elucidate the underlying mechanisms.

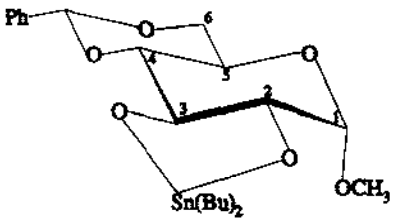
After a brief overview, we shall review the relevant literature concerning several metals and their interactions with mostly monosaccharides and a few oligosaccharides as well as with large polysaccharides. We shall summarize the available spectroscopic, crystallographic and physio-chemical experimental data in attempts to describe binding sites of the metals to carbohydrates. Furthermore, we shall speculate about the compounds and the processes involved, in some biological phenomenon. Finally, we shall make some comments concerning the potential applications of sugar metal complexes.

B. OVERVIEW

(i) Synthetic carbohydrate chemistry

Metals, organo-metallic complexes and metal salts are all widely used in synthetic carbohydrate chemistry. Notably, glycosidic linkages have been formed by reaction of sugar halides with alcoholic acceptors in the presence of heavy metal salts of silver, mercury or cadmium [6,7]. This is an active area of synthetic chemistry but few people have considered the possible metal–sugar interactions [8]. Recent results demonstrate that controlled specific nitrogen coordination by Ag(I) does lead to very high yields in glycosylation reactions [9]. Chemists investigating synthetic carbohydrates also use many of the organo-metallic reagents now available. One example is the use of alkyl stannylenes to activate sugar hydroxyls specifically [10]. For example, the glucose derivative (**V**) reacts with electrophiles regio-selectively at O2 and not O3. The sugar derivatives which react regio-selectively have been shown to form predominantly one dimeric stannylene complex, whereas those derivatives which give mixtures of products form multiple stannylene complexes [11]. This product control can be rationalized from the relative dispositions of the reactive hydroxyl groups. Carbohydrate metal complexes can also be used as chiral auxiliaries.

For example, carbohydrate complexes with titanium have been used to promote enantio- and diastereoselective aldol reactions [12]. Although this type of chemistry has a promising future, it will not be discussed further in this review.



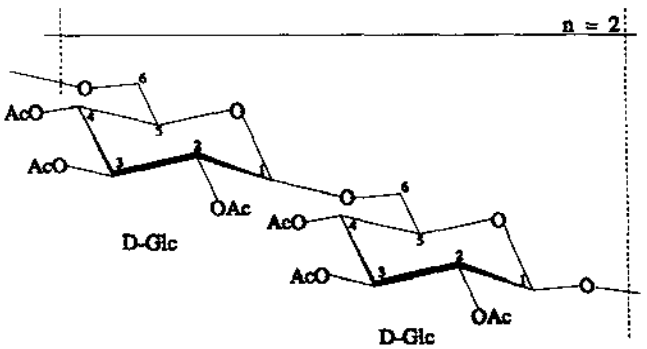
D-Glucose Stannylene Derivative (V)

Before modern spectroscopic methods were applied to carbohydrates, complexation with copper or boron (usually borates) was used to assign relative stereochemistries [13,14]. Arsenate and tellurate complexes that are analogous to the non-metallic borate complexes have been characterized [15]. This type of chemistry is still finding applications but will not be discussed here [16,17].

(ii) Metal complexing carbohydrate derivatives

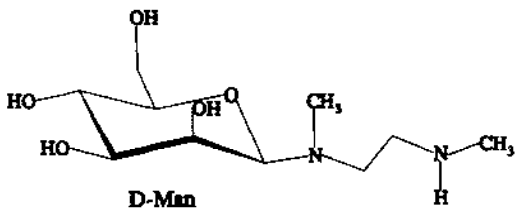
Sugars have well-defined stereochemistries which allow their use as a framework for metal-binding ligands. Notable examples are the incorporation of sugar derivatives into crown ethers [18]. There is one example of a synthetic macrocycle, (β1,6)-cyclogentiotetraose peracetate (**VI**), which complexes alkali metals in a manner analogous to crown ethers [19]. There are, of course, the naturally occurring cyclodextrins such as the (α1,4)-linked hexamers and heptamers of D-glucose (Glc, **VII**). This area has been reviewed recently [20].

Metal binding to synthetic derivatives of carbohydrates has been studied. For



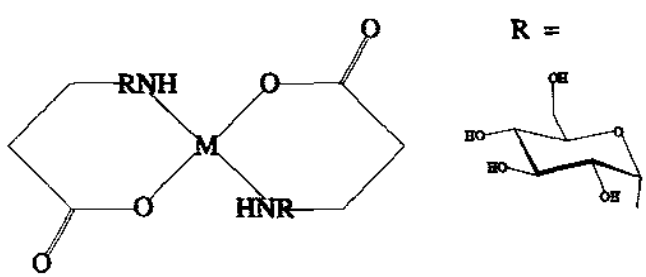
Cyclic Tetramer of Glucose - (β1,6)Cyclogentiotetraose Peracetate (VI)

example, certain salicylaldimine derivatives of D-glucosamine, $GlcNH_2$ (**VIII**), have been shown to bind Cu(II) and Zn(II) [21]. An interesting class of ligands has been developed based on the reaction of ethylenediamine [22] or its C-methyl derivatives [23] with hexoses to give *N*-glycosides which form chiral Co(III) complexes [24]. The analogous Ni(II) complexes derived from Glc have been shown to isomerize to Man by a mechanism which interchanges C1 and C2 [25]. A representative structure is the dimethyl ethylenediamine glycoside of Man (**IX**). Recently, this reaction has been demonstrated to be a general reaction for aldoses leading to epimerization at C2 [26].



N,N'-Dimethyl-Ethylenediamine Glycoside of D-Mannose (IX)

More biologically relevant complexes have been prepared by reacting amino acids with carbohydrates. For example, the monosaccharide cysteine compounds, 2-polyhydroxy-4-thiazolidine carboxylic acids, have been shown to bind Pd(II), Cu(II) [27] and Zn(II) [28]. Also, the Amadori compounds, condensation products of α-amino acids and hexoses, have been shown to bind Pd(II), Pt(II) and Cu(II) [29]. These compounds may have physiological significance since the Amadori reaction can occur in the digestive tract and during the cooking of food and hence may affect the bioavailability of trace metals [30] including Zn(II) [31]. Recent in vitro studies of the effect of Cu(II) on the reaction of glycine and Glc (**VII**) have demonstrated both a rate acceleration and complexation by the products, which are known as melanoidins [32]. For each Cu(II) bound to the melanoidins, two hydrogen ions were released, suggesting the importance of carboxylates for binding. The structures of these complexes have not been established.



Square Planar Metal Complexes of the Glycine-Glucose Amadori Product (X)

Many naturally occurring glycosides are used pharmacologically. One example is the glycopeptide antibiotic Bleomycin, which has anti-neoplastic properties. This drug is thought to function by complexing Fe(II), which initiates O2' of (**IV**) activation and DNA breakage. In this case, the carbohydrate is thought to help stabilize the O2' scission [33]. In vitro studies of such $OH^{\bullet}$-induced damage to nucleic acids have demonstrated the importance of considering Fe binding to the Rib among other factors [34]. However, for the purpose of this review we will concentrate on carbohydrates without chemical derivitization such as those typically found in nature.

(iii) Conformations of carbohydrates

It is the conformations of carbohydrates that to a large extent determines their ability to form complexes and influences the chemistry of the complexes [35]. The conformational analyses of carbohydrates has one unique characteristic which is that the absolute stereochemistry at every chiral centre is known a priori, by simply knowing which sugar is being used, D or L, α or β, gluco or manno, etc. [36]. This knowledge, coupled with ^{1}H NMR spectral data such as dihedral angle-dependent coupling constants and inter-proton distance-dependent nuclear Overhauser enhancements (NOEs), often leads to a good description of solution conformations for monosaccharides [37,38].

This type of analysis has been widely used by Angyal [39] to derive rules for predicting metal complexation capacities of neutral sugars. Briefly, for six-membered rings the axial, equatorial, axial (a,e,a) disposition and the 1,3,5 triaxial conformation are the best complexing arrangements (see Fig. 2). Both cis and trans diols in six-membered rings can complex, but only cis diols in five-membered rings can. For open chain systems, this translates into a preference of threo > erythro (t > e) for diols and tt > te > ee for triols (see Fig. 3). Virtually all of these studies have been done with monosaccharides and almost no data are available for oligosaccharides. Consequently, there are only a couple of examples of chelation involving two monosaccharide units from the same oligosaccharide [40,41].

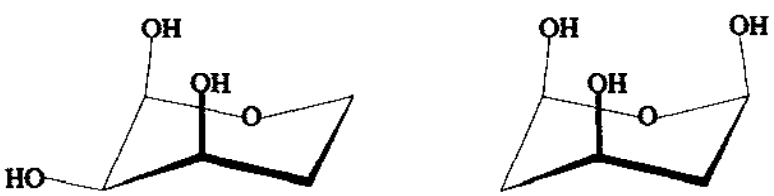


Fig. 2. Conformations of six-membered ring polyols which are favourable for metal complexation.

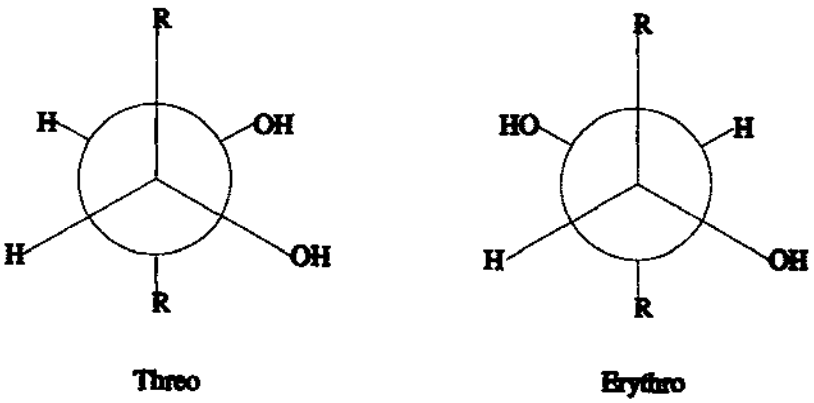


Fig. 3. Newman projections of threo and erythro diols in their lowest energy conformations. The gauche hydroxyls of the threo diol can coordinate metals better than the trans hydroxyls of the erythro diol.

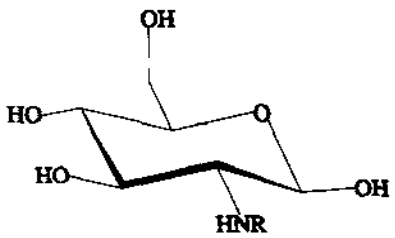
(iv) Role of the metal

These carbohydrate stereochemical rules serve as guidelines. Metals are all unique and such important factors as bond lengths and coordination geometries need to be considered. The examples discussed by Angyal all involve neutral oxygen donor sites and hence form only weak complexes. In a recent review, he has compiled the known binding constants to neutral sugars [42] (see also a brief review in ref. 43).

Strong cation complexation involves other binding sites as discussed by Burger and Nagy [44]. In sugars, these can be amides, amines, carboxylates as well as phosphate and sulfate esters [45]. Metal complexation to the amide (usually acetamide) groups of sugars has only been demonstrated using lanthanides [46] and is a weak interaction [47]. This may in part relate to the steric congestion about most sugar amide groups. Binding to hexosamines has been studied and leads to moderately strong complexes with Ni(II), Cu(II) and Co(II), as is to be expected with amine ligands [48,49]. Underivatized hexosamines are only rarely found in nature. The notable examples are the glycosyl inositol phosphatidyl anchors [50], which have an invariant $GlcNH_2$ α-linked to the inositol [51,52]. Metal binding to these structures has not been considered to our knowledge.

The carboxylic acid group, on the other hand, is known to be a good metal binding group and in fact a number of complexes have been reported and will be discussed below [53]. In addition, phosphate esters are known to be good binding sites, but to our knowledge metal binding to sugar phosphates has not been studied. Metal–phosphate complexes may have relevance to the biosynthesis of carbohydrates since the sugar donors are often nucleotide phosphate diesters and many of the transferase-catalysed reactions have strict metal ion requirements [54]. In fact, nucleotides are known to have critical interactions with Mg(II), Mn(II), etc. [55].

One could also include the polynucleic acids DNA and RNA in this discussion since they have a sugar backbone and have been investigated for metal complexation



R = H is 2-amino-2-deoxy-ß-D-glucopyranose (VIII) and
R = $COCH_3$ is 2-acetamido-2-deoxy-ß-D-glucopyranose (XI)

[56]. Indeed, metals such as Fe(III) and Cu(II) can not only bind to DNA and induce conformational changes, but via free radical chemistry lead to DNA damage [57]. Most metals will interact with the nucleotide bases or the phosphate groups with only a few examples such as *cis*-Pt(ND_2CH_3) which chelate both [58]. Ni(II) has been shown to bind to both the adenosine base and the phosphate groups of ATP in aqueous solution by a neutron diffraction technique [59]. However, it is only with metals which readily form oxo-cations, such as vanadium and molybdenum, that strong complexation to the sugar residues has been established [60]. Also well known are *O*-sulfates and *N*-sulfates, but the possible role of these groups as metal binding sites has not been clarified. This is an active area of research in our group [61].

Thus, our focus will be on acidic sugars containing carboxylates and sulfates since these are the ones that have been most studied. These share the common property of being oxygen-based ligands. Figure 4, taken from the work of Martell and co-workers, demonstrates a linear relation between the log of the hydroxide binding constant versus the log of the oxygen-containing ligand binding constant, for a series of metals [62]. Those at the upper right showing the highest constants include Fe(III) and Cu(II). This prediction will be seen to be satisfied by experiment since, for the most part, Fe(III) and Cu(II) show the most significant interactions with carbohydrates.

These functional groups are also those present in the biological macromolecules known to be involved in metal metabolism in the biosphere [63]. Another important aspect of metal binding to such carbohydrates is the ionic radii of the metals, which presumably will influence their ability to be accommodated by the ligand. For example, the ionic radii for some metals are Cu(II): 0.87 Å; Ca(II): 1.14 Å and La (III): 1.22 Å [64–66]. We will present data on binding to small oligosaccharides and to biopolymers. The presentation is conveniently broken down into alkali metals, alkaline earth metals, notably calcium, and rare earth metals. Then the individual metals molybdenum, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc and lead followed by some examples with other metals.

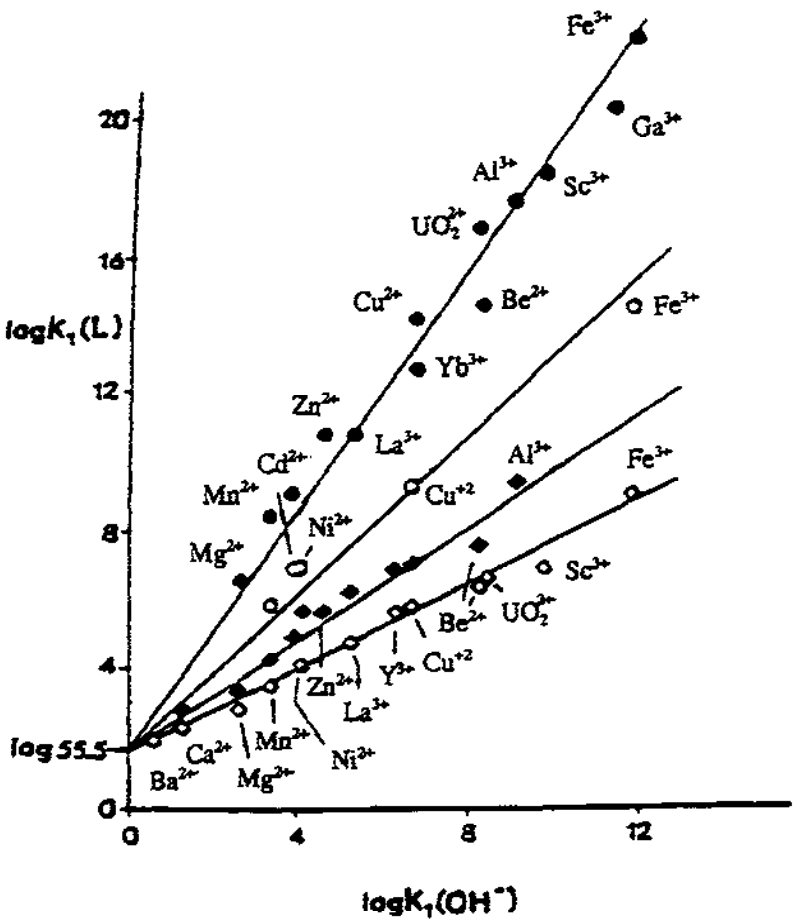


Fig. 4. Log K_1 values for catecholate (●), 5-nitrosalicylate (○), kojate (◆) and malonate (◇) versus log K_1(OH$^-$) values for the metal ions. The log 55.5 intercept is the theoretical value of the entropy contribution to the chelate effect on the basis of the asymmetry of the standard reference state [350,351]. Figure reproduced with permission.

C. SPECIFIC EXAMPLES

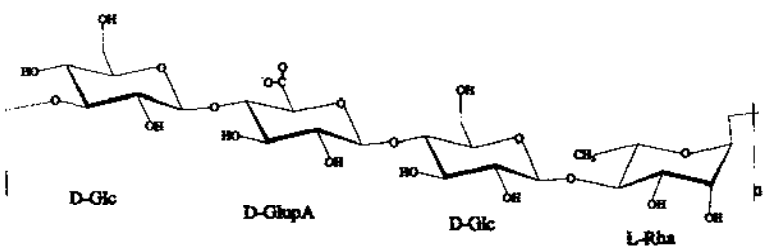
(i) Alkali metals

The work of Lein and Cram [67], Pedersen [68] and Lehn [69] with synthetic ligands has shown that, in order to bind alkali metals, a preorganized ligand with multiple binding sites is necessary. With the exceptions of the macrocycles (**VI**) described above, pre-organized oligosaccharides have not been specifically synthesized. All alkali metal complexes described to date are very weak [70]. Another important factor is the competition between ligand solvation and cation solvation, which will be a decisive factor for aqueous solutions of carbohydrates [71].

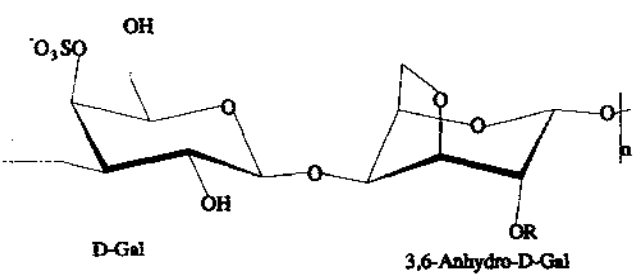
In fact, most information about alkali-metal coordination has been obtained

from cystallographic studies in which salts have been co-crystallized with the sugars [72]. The typical procedures involve slow evaporation of aqueous or water/alcohol solutions of the sugar and the metal salt [73]. In these cases, it is not surprising that sugar hydroxyls will replace the displaced waters of hydration. Similar crystallization methods have been applied to the alkali metal salts of sugar acids and indeed coordination to the carboxylate [74] or the sulfate has been observed [75]. Such studies have helped to develop models of carbohydrate and metal salt hydration.

Alkali metals do have a pronounced effect on charged carbohydrate polymers [76–78]. Figure 5 shows structures of some of these compounds including: gellan gum (**XII**) from a microbial source, carrageenan (**XIII**) from seaweed, pectin (**XIV**) and alginate (**XV**) from plant cell walls and hyaluronate (**XVI**), chondroitin sulfate (**XVII**) and heparin (**XVIII**), which are glycosylaminoglycans from mammalian sources. Most of these alkali-induced effects involve "screening" of the negative charges of the polymer which in turn can lead to conformational changes in the polymer and hence functional changes. The cation-induced gelling properties have been studied in detail. The existence of both inter- and intra-chain carbohydrate:cation interactions have been demonstrated [79]. This type of alkali salt-induced conformational change has been investigated in polymers with only carboxylates as charged groups, i.e. hyaluronate [80–82] and xanthan [83], in polymers with sulfate

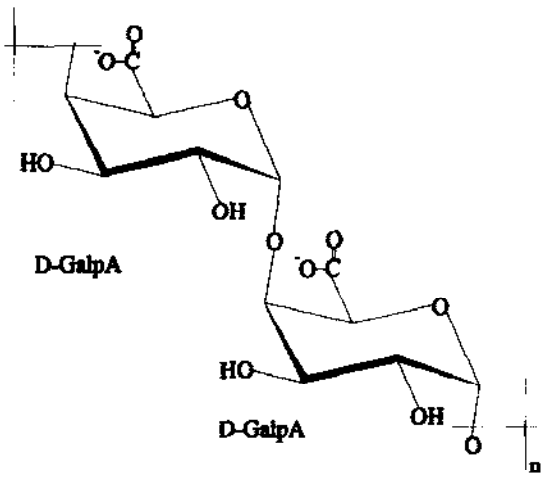


Bacterial Polysaccharide Gellan (**XII**)

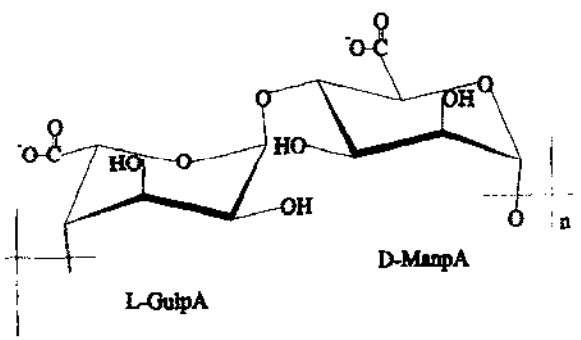


R = H or SO_3^-

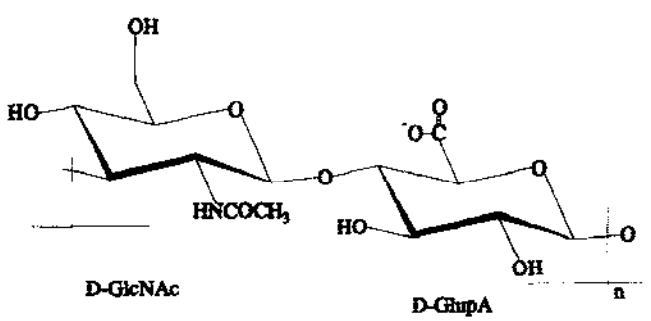
Sulfated Polysaccharides Carrageenans (**XIII**)



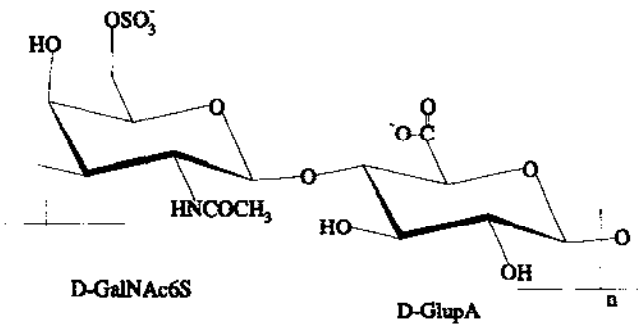
Plant Cell Wall Polysaccharide Pectin (XIV)



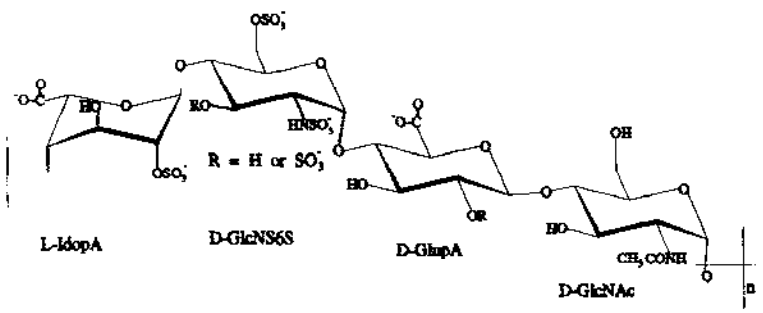
Plant Cell Wall Polysaccharide Alginate (XV)



Hyaluronic Acid (XVI) a Mammalian Polysaccharide with Carboxylates



Chondroitin Sulfate (XVII) a Sulfated Mammalian Polysaccharide



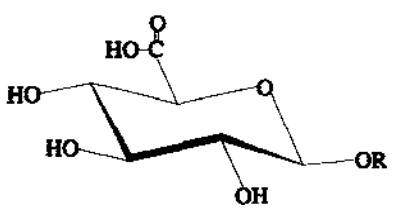
Representative Structure of Heterogeneous Mammalian Polysaccharide Heparin (XVIII) with both Sulfates and Carboxylates

Fig. 5. Representative chemical structures of some carbohydrate biopolymers: gellan gum (XII) from a microbial source, carrageenan (XIII) from seaweed, pectin (XIV) from fruits and alginate (XV) from plant cell walls, and hyaluronate (XVI), chondroitin sulfate (XVII) and heparin (XVIII) from mammalian sources. Note that the patterns of sulfation for chondroitin sulfate and heparin add additional heterogeneity as does the relative abundance of IdopA versus GlupA residues for heparin.

esters as the charged groups such as carrageenans [84,85] and in polymers which have both sulfates and carboxylates such as heparins [86]. For example, the effect of different alkali cations on the helix-to-coil transition of carrageenans has been quantified using variable temperature IR spectroscopy [87].

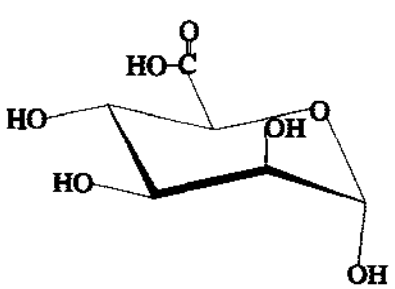
In these studies, different properties of the complexes have been measured using the appropriate techniques: isotherms from equilibrium dialysis [88], self-diffusion coefficients using a capillary method with radioisotopes [89], activity coefficients from ion selective electrodes [90], enthalpies from calorimetry [91], CD of the polymer [92], the degree of water binding to the polymer by IR spectroscopy [93] and the degree of desolvation by NMR spectroscopy of the counterion [94]. ^{23}Na or ^{87}Rb NMR are the most widely used and changes in linewidths (T_2) or T_1 are frequently monitored [95,96]. The amount of associated ion and the degree of desolvation can be estimated from these studies, assuming models for the particular system. Most groups concur that no specific binding is observed with mono-valent cations.

This concept of charge screening by counterions to charged polymers in aqueous solutions is well established and has been termed the "polyelectrolyte effect" by Manning [97]. The result of this effect is that counterions accumulate adjacent to the charged polymer by electrostatic interaction without substantial desolvation. This type of association has been termed "territorial" binding as opposed to "site" binding. The concept of territorial binding predicts that the amount of territorial

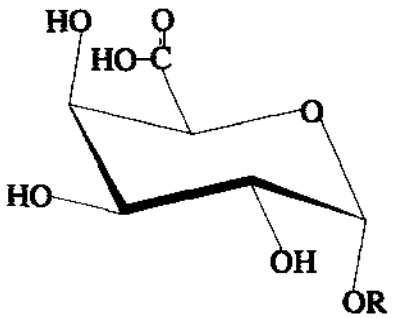


R = H ß-D-Glucopyranosiduronic Acid (**XIX**) and

R = CH_3 Methyl ß-D-Glucopyranosiduronic Acid (**XXII**)

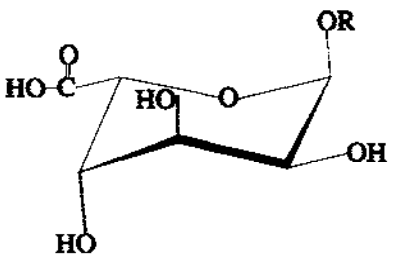


α-D-Mannopyranosiduronic Acid (**XX**)



R = H α-D-Galactopyranosiduronic Acid (XXIII) and

R = CH_3 Methyl α-D-Galactopyranosiduronic Acid (XXIV)



R = H α-L-Gulopyranosiduronic Acid (XXV) and

R = CH_3 Methyl α-L-Gulopyranosiduronic Acid (XXVI)

Fig. 6. Chemical structures of some uronic acids: R = H, β-D–glucuronic acid (**XIX**) and R = CH_3 its methyl glycoside (**XXII**), α-D-mannuronic acid (**XX**), R = H, α-D-galacturonic acid (**XXIII**) and R = CH_3 its methyl glycoside (**XXIV**), and α-L-guluronic acid (**XXV**) and R = CH_3 its methyl glycoside (**XXVI**).

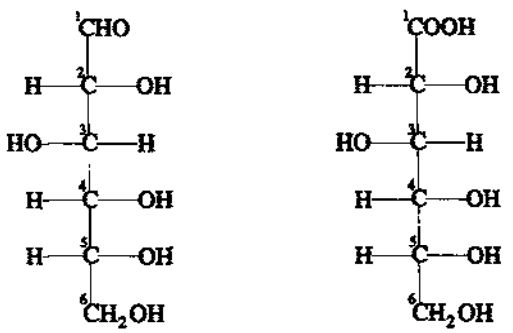
binding depends on the ion valence squared times the aqueous ion activity, leading to preferential accumulation of higher valent ions adjacent to the polymer [98], as well as a dependence on the charge of the polymer. Both of these predictions were substantiated by experiment using carbohydrate polymers [99].

(ii) Alkaline earth metals including calcium

Calcium is the most studied of the metals known to bind to carbohydrates. This interest stems from the known affinity for oxygen-based ligands, especially carboxylates, for Ca(II), as well as from many observations of Ca(II)-induced changes in the physical state of carbohydrate solutions. For example, heparin and related polymers are known to inhibit $CaCO_3$ (calcite) crystallization. These observations form the basis of a hypothesis that removal or degradation of the polymers may be a prerequisite for the in vivo formation of pathological biomineralizations [100]. Furthermore, calcium:carbohydrate complexes appear to participate in a variety of biological processes such as calcium storage, calcification and calcium-dependent

cell-cell interactions [101–103]. Other examples include Ca(II)-mediated carbohydrate–protein binding [104] and Ca(II) interactions with glycolipids [105]. The most important commercial application is the Ca(II)-induced gelling of polysaccharides which is widely used in the food industry to produce ice cream, jams, salad dressings, etc. [106,107].

Most of these interactions involve carboxylic acid derivatives of sugars. The commonest are the uronic acids which are the terminal C6 oxidized aldoses. Therefore, D-glucuronic acid (**XIX**) is the uronic acid from D-glucose (**VII**), D-mannuronic (**XX**) from D-mannose (**III**), etc. Also frequently encountered are the aldonic acids which are the C1 oxidized aldoses, such as D-gluconic acid (**XXI**). This is the basis of the sugar diagnostic test, the Fehlings reaction, in which, for example, Glc is oxidized by Cu(II) to GlcA. Since the pK_A values are typically around 3.2 for these sugar acids, at neutral pH they are found as salts [108]. Figure 6 shows some representative structures of uronic acids. Keto-acids such as the sialic acids are common components of animal cell walls. The prototype sialic acid is 5-acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid (*N*-acetyl neuraminic acid, NeuNAc, **XXVII**). Its association with calcium prompted one group to title their paper "Sialic acid — a calcium binding carbohydrate" [109].



Fisher projections of D-Glucose (**VII**) and D-Gluconic Acid (**XXI**)

Calcium(II) binding to neutral sugars, notably Rib, in aqueous solutions was studied by observation of specific downfield shifted hydroxyl proton resonances in the presence of $CaCl_2$, in the ^1H NMR spectra. Binding affinities followed the ax–eq–ax rules discussed above [110,111]. Thermodynamic binding constants in terms of pair interaction parameters for several neutral sugars have been determined electrochemically. For example, Rib and Ca(II) gives values of -2.3 compared with -0.1 kJ kg mol^{-2} with K(I) [112]. The involvement of the side chain of NeuNAc in calcium binding was shown by NMR [109] as well as a preference for the β-anomer [113]. Binding constants were determined to be 193 versus 121 M^{-1} for *N*-glycoyl (**XXVIII**) versus *N*-acetyl (**XXVII**) neuraminic acid by NMR [114] and for NeuNAc by potentiometry [115]. These values compare with about 5 M^{-1} for Ca(II) and GlupA or GalpA (Murexide titrations) [116] or 33 and 72 M^{-1} (Ca(II) or H^+

titrations) [117]. The higher constants for NeuNAc reflect the involvement of the side chain. These observations are consistent with solubility measurements of $CaSO_4$ in sugar solutions that found binding strengths decreasing in the order GlcA > lactate > acetate > glucitol > glycerol [118]. Binding constants are compiled in Table 1 along with more details of the experimental conditions.

Although these numbers are small, it seems likely that most sialic acids exist in Nature as Ca(II) complexes, since Ca(II) concentrations are in the 1–10 mM range and virtually all of the sialic acid is located on the outer surface of the cell. Gangliosides are sialic acid-containing glycolipids found on virtually all cells, but preferentially in the brain [119]. Early work, mainly by Probst et al. [120,121] suggested that they have a physiological role in neural transmission by proposing a calcium serotonin exchange mechanism [122,123]. This assertion was supported by large association constants (log K about 4) for Ca(II) and various gangliosides [124]. These glycolipids, due to their amphiphatic nature, must be studied in micelles or bilayers. Since they are negatively charged, the micelles and/or bilayer exhibit negative surface potentials and cation adsorption similar to polyelectrolytes discussed above [125–127]. Calculations based on the Gouy–Chapman–Stern model [128] estimate intrinsic binding constants to be $<100\ M^{-1}$ for Ca(II)–ganglioside complexes while still in agreement with the marked conductance and elecrophoretic changes found experimentally.

Alternatively, Ca(II) binding has been studied by the preparation of solid complexes with Ca(II), Mg(II), Sr(II) and Ba(II), and various monosaccharides [129,130]. IR spectroscopy of these complexes indicates that waters of hydration remain in the solid state although usually with rearranged H-bonding patterns from the metal-free sugars. Also in cases of the uronate salts, definite evidence for carboxylate-metal cation interactions comes from changes in the carboxylate stretching bands [131,132].

The most informative results come from crystallographic studies. Details about the crystal structures of Ca(II)–carbohydrate complexes have been previously reviewed [133–135]. Coordination numbers are high, varying from 7 to 9. Typically, two or three sugar oxygens come from one saccharide, and consequently two or more carbohydrates frequently bind one Ca(II) ion. Almost invariably the structures are hydrated, frequently with one or more Ca(II)-coordinated moleclues of water. For instance, in [α-D-glucuronate · $CaBr \cdot 3H_2O$], the Ca(II) ions is coordinated by three monosaccharides [136]. One monosaccharide provides its carboxylate oxygen and its ring oxygen (O5) for bonding, the second supplies its carboxylate oxygen, and its O4 for binding and the third has its O1 and O2 bound (see Fig. 7). Two molecules of water also coordinate the Ca(II) to make a total coordination number of eight. Calcium–oxygen bond lengths range from 2.384 to 2.567 Å. It should also be noted that the carboxylate oxygen atoms and O5 are nearly co-planar (O6C6–C5O5, 2.9°).

The investigations of the mechanism of polysaccharide gel formation have led

TABLE 1

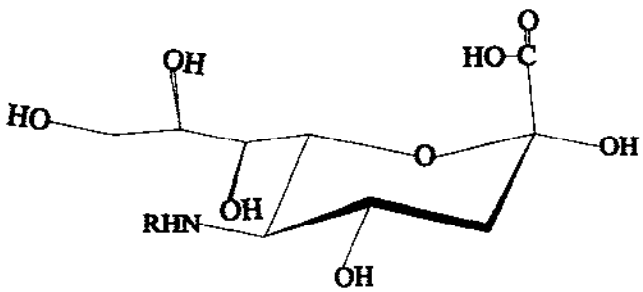
Stability constants of various metals with carbohydrates

Metal	Sugar	β_{1mp}		Technique and conditions	Ref.
Ca(II)	GlupA (**XIX**)	110	1.51		117
	αMeGlupA	110	1.60	Ca(II) I.S.E. and H^+ potentiometry	
	GalpA (**XXIII**)	110	1.81	$I = \rightarrow 0$, $T = 25°C$	
	βMeGalpA (**XXIV**)	110	1.75		
Ca(II)	GalpA (**XXIII**)	110	0.70		116
	GlupA (**XIX**)	110	0.72		
	GlcA (**XXI**)	110	1.15		
	Heparin (**XVIII**)	1:1[a]	2.09	Ca–Murexide spectrophotometry	
	Chondroitin Sulfate A (**XVII**)	1:1[a]	1.24	$I = 0.15$	
	Chondroitin Sulfate B (**XVII**)	1:1[a]	1.39		
Ca(II)	NeuNAc (**XXVII**)	110	1.9	Ca(II) I.S.E. potentiometry $I = 0.02–0.04$, $T = 25°C$	115
Ca(II)	NeuNAc (**XXVII**)	110	2.08	^1H NMR	114
	NeuGc (**XXVII**)	110	2.29	^1H NMR	
Ga(II)	GlcA (**XXI**)	110	0.41	Solubility measurements $I = 0.2–0.8$, $T = 25°C$	118
Mg(II)	NeuNAc(**XXVII**)	110	1.5	Ca(II) I.S.E. potentiometry $I = 0.02–0.04$, $T = 25°C$	115
Nb(V)	GlcA (**XXI**)	110	2.78	H^+ potentiometry	329
Eu(III)	GalpA (**XXIII**)	110	1.81	H^+ potentiometry	248
	GlupA (**XIX**)	110	1.60	$I = 1.0$, $T = 25°C$	
Eu(III)	αMeGalpA (**XXIV**)	110	0.74	L.I.S. ^1H NMR	166
		210	2.20		
		310	2.54		
VO^{2+}	GlupA (**XIX**)	120	3.90	H^+ potentiometry $I = 0.1$, $T = 25°C$	207
VO^{2+}	Lactobionic (**XXXVIII**)	120	6.07	H^+ potentiometry $I = 0.1$, $T = 25°C$	210
Fe(II)	GalpA (**XXIII**)	110	3.09	H^+ potentiometry	235
		120	5.58	$I = 0.01$, $T = 25°C$	
Fe(III)	GalpA (**XXIII**)	130	8.51		235
Co(II)	βMeGlcNH$_2$	110	2.93	H^+ potentiometry	247
	GlcNH$_2$ (**VIII**)	120	4.09	$I = 0.15$, $T = 25°C$	
	GalNH$_2$ (**XXXIII**)	120	6.50		
Ni(II)	βMeGlcNH$_2$	110	3.10		247
	GlcNH$_2$ (**VIII**)	210	6.43	H^+ potentiometry	
	GalNH$_2$ (**XXXIII**)	210	5.96	$I = 0.15$, $T = 25°C$	
	ManNH$_2$	210	6.11		
Ni(II)	GlcA (**XXI**)	110	1.82		297

TABLE I (*continued*)

Cu(II)	βMeGlcNH$_2$	110	4.13	H$^+$ potentiometry	247
		120	7.52	$I=0.15$, $T=25$°C	268
	GlcNH$_2$ (**VIII**)	110	~(5.12)		269
		120	9.02(8.85)	polarography	
	GalNH$_2$ (**XXXIII**)	110	4.20(5.23)	$I=0.15$, $T=25$°C	
		120	9.13(9.02)		
	αMeGalNH$_2$	110	4.40		
		120	8.40	H$^+$ potentiometry	
	ManNH$_2$	120	9.68	$I=0.15$, $T=25$°C	
	αMeManNH$_2$	110	4.81		
Cu(II)	GalpA (**XXIII**)	210	3.0	H$^+$ potentiometry	217
				$I=1.0$, $T=25$°C	
Cu(II)	GalpA (**XXIII**)	110	3.39	H$^+$ potentiometry	235
		210	5.99	$I=0.01$, $T=25$°C	
Cu(II)	GalpA (**XXIII**)	110	2.16	E.P.R, Cu(II) potentiometry	273
		210	2.05	$I=1.0$, $T=25$°C	
Cu(II)	GalpA (**XXIII**)	110	1.81	H$^+$ potentiometry	248
	GlupA (**XIX**)	110	1.48	$I=1.0$, $T=25$°C	
Cu(II)	GlupA (**XIX**)	110	1.01	^{13}C NMR	274
		210	4.1		
Cu(II)	GlupA (**XIX**)	110	1.48	Calorimetry	275
	GalpA (**XXIII**)	110	1.81	$I=1.0$, $T=25$°C	
Cu(II)	Lactobionic (**XXXVII**)	120	5.46	Polarography $I=0.1$, $T=25$°C	210
Cu(II)	Starch 2,3-Dicarboxylate	1:1[a]	4.32	Cu(II) potentiometry $I=0.15$, $T=25$°C	355
Zn(II)	MeIdopA (**XLI**)	110	2.25	^{1}H NMR	61
Zn(II)	IdopA2S (**XLIIa**)	110	2.67	^{1}H NMR	311
Zn(II)	IdopA2S (**XLIIb**)	110	2.84	^{1}H NMR	
Zn(II)	IdopA2S (**XLIIIa**)	110	3.94	^{1}H NMR	
Zn(II)	IdopA2S (**XLIIIb**)	110	4.30	^{1}H NMR	
Zn(II)	Pectin (**XIV**)	1:1[a]	4.56	Spectrophotometry $I=0.02$, $T=25$°C	271
Cd(II)	GalpA (**XXIII**)	110	1.15	H$^+$ potentiometry	248
	GlupA (**XIX**)	110	1.10	$I=1.0$, $T=25$°C	
Pb(II)	GalpA (**XXIII**)	110	2.00	H$^+$ potentiometry	248
	GlupA (**XIX**)	110	1.62	$I=1.0$, $T=25$°C	
Pb(II)	GlcA (**XXI**)	110	2.13	H$^+$ potentiometry	316
		210	3.35	$I=1.0$, $T=25$°C	
Pb(II)	GalpA (**XXIII**)	110	2.51	Pb(II) potentiometry $I=0.01$, $T=25$°C	314

[a] Molarity based on disaccharide repeat units of polymers.



R = $COCH_3$ N-Acetyl Neuraminic Acid (**XXVII**) and R = $COCH_2OH$ N-Glycoyl Neuraminic Acid (**XXVIII**)

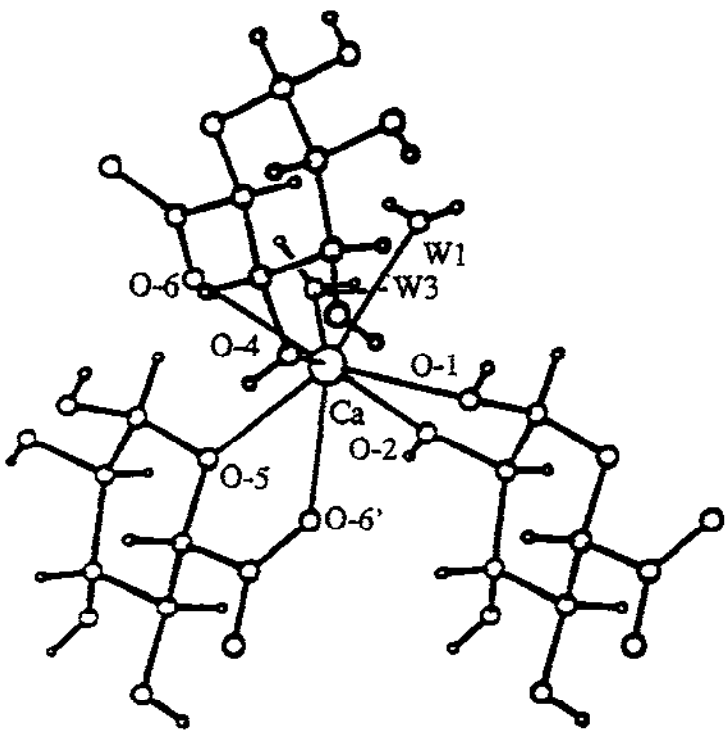


Fig. 7. Environment of the calcium ion, which is coordinated to three D-glucuronate anions and to two water molecules. W1 and W3 correspond to oxygen atoms of water molecules. Figure reproduced with permission.

several groups to study interactions of Ca(II) with oligomers (typically DP 1–6) of the biopolymers, usually made available by degradation and purification of the intact polymer. For example, Rinaudo et al. have studied Ca(II) binding to oligogalacturonates (DP 2–5) by NMR. The ^{1}H and ^{13}C NMR spectra are not noticeably affected

below 0.3 equivalents of Ca(II) per carboxylate. Above this concentration, a rigid gel is formed and the NMR signals effectively disappear. Other physical parameters, such as the viscosity and the osmotic coefficient, also show the same concentration dependence. These data indicate that the gel formation entails a cross-linking mechanism that involves Ca(II) ions, possibly via carboxylate–calcium–carboxylate bridges [137]. The interaction of Ca(II) with intact polymers has been studied by IR spectroscopy [138], NMR relaxation measurements [139], calorimetry [140], potentiometry [141] and by activity coefficient measurements using potentiometry and the indicator tetramethylmurexide [142]. The last paper clearly shows a relationship between Ca(II) binding and polymer charge density.

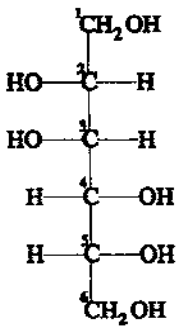
Equilibrium dialysis studies have shown that heparin binds Ca(II) better than the other glycosylaminoglycans [143]. Similar studies, also using NMR and CD, have shown that a cooperative conformational transition is induced by Ca(II) with about one Ca(II) bound per tetrasaccharide unit [144]. A specific site for Ca(II) binding could not be established by studying heparins with different compositions [145]. In fact, both the NMR data [146,147] and Ca(II) ion activity data can be explained by suitable models of the Manning theory [148]. Thus, it is the high charge density of heparin which largely dictates these interactions.

These Ca(II) heparin interactions are not without importance as Ca(II) is known to modulate the pharmacological affects of heparin [149]. For example, heparin has been shown to release Ca(II) from stores in the sarcoplasmic reticulum of muscle cells [150]. However, pharmacological doses of heparin do not seem to change the serum Ca(II) levels during surgery [151]. All of these effects are likely related to the charge density of heparin and its apparent changes after Ca(II) condensation.

A study by Ricard and co-workers has shown that metal cations, particularly Ca(II), modulate the activity of the enzyme pectin methylesterase not by interacting with the protein but by complexation to the pectin [152]. This enzyme controls the degree of esterification and hence the surface potential of the plant cell wall. The growth rate of the plant is directly affected by the surface potential as is metal binding. Thus metal binding to pectin is a control on plant growth [153].

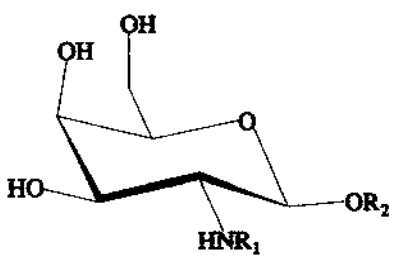
(iii) Rare earth metals

The determination of solution structures of carbohydrates involves lanthanide shift reagents. These reagents have been used as probes of calcium binding carbohydrates [154]. Eu(III), Pr(III) and Gd(III) are the most widely used NMR probes [155]. ^{1}H [156] and ^{13}C [157] NMR studies of lanthanide induced shifts of alditols confirm the conformational preferences discussed in the overview. For example, the coupling constants particularly (J_{23}) of mannitol (**XXIX**) change in the presence of lanthanum, indicating that a conformational change occurred. Studies of molecular models demonstrates that mannitol must substantially reorient in order to obtain the geometry required for metal binding. Consequently, mannitol is one of the



Fisher Projection of the Alditol D-Mannitol (**XXIX**)

weakest binders. Lanthanide-induced shifts have been used to interpret ^{1}H and ^{13}C NMR data of 2-acetamido derivatives of methyl-D-gluco and D-galactopyranosides (MeGlcNAc, **XXX** and MeGalNAc, **XXXI**) in the presence of lanthanide ions [45]. The data suggest that lanthanide ions form a complex with the acetamido group. Lanthanides binding to neutral sugars [158] and a glycopeptide [159] have also been reported.



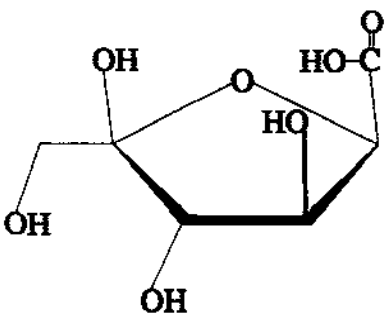
$R_1 = COCH_3$, $R_2 = CH_3$ Methyl-ß-D-2-acetamido-2-deoxy-Galactopyranose (**XXXI**)

$R_1 = COCH_3$, $R_2 = H$ ß-D-2-acetamido-2-deoxy-Galactopyranose (**XXXII**)

$R_1 = R_2 = H$ ß-D-2-amino-2-deoxy-Galactopyranose (**XXXIII**)

The interactions between lanthanide ions and uronic acids have been investigated in a number of studies. For example, in a study of the influence of some paramagnetic lanthanide ions on the NMR spectra of methyl-L-gulopyranoside (MeGulpA, **XXVI**) it was shown that a 1:1 complex was formed [160]. Another report, which compares NMR spectra of a complex between Gd(III) and D-xylo-5-hexulosonic acid (5-oxo-D-gluconic acid, **XXXIV**) in the solid state and in solution, shows that in the solid state structure the β-furanose form is found but in solution the α-furanose form is present [161]. Solution complexes between GlupA and Ce(IV) [162] have been reported.

Angyal et al. [163] have suggested that the carboxylate oxygen (O6) and O5 of the α-anomers of GlupA and GalpA chelate, whereas in the β-anomers O5 is not involved. A similar conclusion was reached by Izumi [164], using ^{13}C NMR spectra



α-D-Xylo-5-Hexulosonic Acid (XXXIV)

of sodium GlupA and GalpA and the shifts induced by lanthanide cations in aqueous medium. It was found that in the α-anomers, a cation is close to the O5 ring oxygen and to one carboxylate group (O6), but in the β-anomers it is not close to O5 and is coordinated only to one (or both) of the carboxylate oxygen atoms. Similarly, in the Eu(III) interaction with α-D-GalpA described by Anthonsen et al. [165], the axial hydroxyl at C4 (O4) acts in concert with the equatorial carboxyl group and the ring oxygen (O5) as the specific binding sites. Interestingly, all these studies have indicated that the O5 ring oxygen is involved in binding. Subsequent reports determined the values for La(III) binding to the α-methyl glycoside of GalpA as 350 M^{-1} (1:3), 160 M^{-1} (1:2) and 6.5 M^{-1} (1:1) for La(III) [166].

These studies of uronic acids were motivated by the need to understand metal binding to polysaccarides. De Bolster and co-workers [167] have developed a model of the binding of Yb(III) to chondroitin sulfate based on a comparison to the X-ray crystal structures of the Ca(II) and Na(I) forms of chondroitin sulfate [168,169]. This model is based on cation binding to the carboxylates and the ring oxygen as shown in Fig. 8. They find little evidence for binding to the O-sulfate groups. La(III) binding to acidic polymers has also been used to confirm the valence dependence of the Manning theory [170].

(iv) Molybdenum and vanadium

Molybdenum, as a micronutrient trace element, is distributed widely in nature and plays an important role in plant and animal nutrition. Perhaps one of the most interesting aspects of molybdenum is the role it plays in biological processes. Much of the work on the biological function of the molybdenum has been stimulated by its presence in soils [171–174]. Moreover, it has been identified as a co-factor in a variety of bacterial, plant and mammalian enzymes [175,176]. The most important molybdenum enzymes are nitrogenases [177,178], which reduce molecular nitrogen

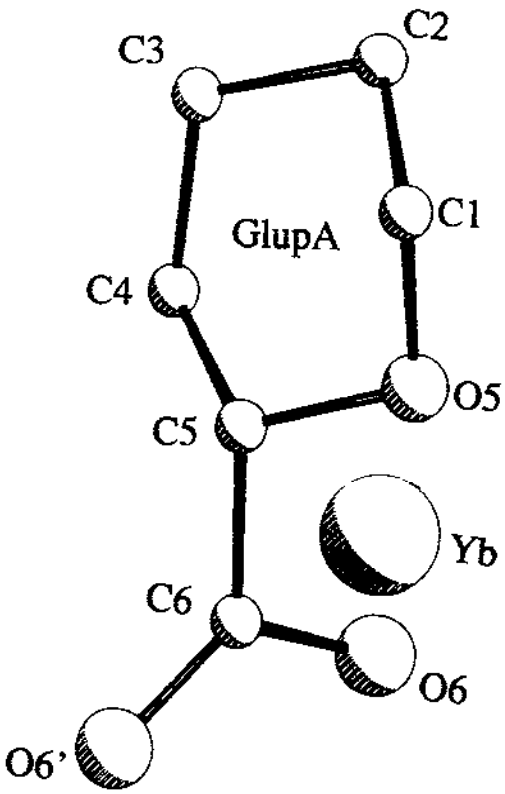


Fig. 8. Structure of ytterbium β-D-glucuronate in solution, from ^{13}C NMR data; a 1:1 complex is assumed. Figure reproduced with permission.

to ammonia. Vanadium is also present in nitrogenases of some fixing bacteria [179] and is detected in various species of mushrooms [180].

Most bacteria produce extracellular polysaccharides under specific conditions. They may take the form of discrete capsules or may be free of any attachment to the cell and are then described as "slime". The exact state of the polysaccharide seems to depend on the particular bacterial species and the age and condition of the culture [181]. Little is known about the biological roles or functions of the extracellular polysaccharides, but many have regular structures [182] that are related to polysaccharides from plant [183,184] and animal [185] sources. The bacterial cell wall is composed of layers of peptidoglycans, where the outer layer contains proteins, polysaccharides and lipids. These polysaccharides that lie outside the cell wall are called capsular polysaccharides. The role of the capsule is not clear, but in some pathogens it is suggested that the capsule functions as a protective layer to the organism. It appears that the capsules have the capacity to adsorb or bind metals in the outer layer of the cell. Gram-negative bacteria have the largest amount of polysaccharide at the root surfaces [186,187]. The binding of molybdenum by

Rhizosphere bacteria, in particular to the well-defined strain of *Pseudomonas aeruginosa* has been investigated [188,189]. It was speculated that the involvement of extracellular polysaccharides produced by *Pseudomonas aeruginosa* bound molybdenum and thereby reducing the availability of molybdenum as a micronutrient to plants. It was suggested [190,191] that the uronic acid residues in the polysaccharide were responsible for the binding of molybdenum.

Aqueous molybdenum chemistry is complicated since a number of oxidation states are known and since molybdenum forms oxo-cations [192]. Hence, we investigated the interaction of the Mo(VI) oxo-cation $[Mo_2O_5]^{+2}$ with uronic acids in aqueous solution by ^1H and ^{13}C NMR spectroscopy and found that complexation of GlupA involves the carboxylate oxygen O6 and O4 of the hydroxyl oxygen in a pyranose form $^4C_1$ in the pH region 3.5–5.8 (see Fig. 9) [193]. Other studies on Mo(VI) with uronic acids in the solid state by IR, X-ray photoelectron spectroscopy and magnetic moment data suggested the presence of oxygen bridges producing diamagnetic properties [189]. Other ^1H and ^{13}C NMR studies on the interaction of molybdenum oxo-cations with monosaccharide related compounds have been reported [194–196]. An unusual rearrangement mechanism of aldoses which involves a molybdate-catalysed inversion has been described and used to enrich monosaccharides with oxygen isotopes useful for metabolic tracers [197,198]. Two groups have investigated the mechanism of this reaction and both conclude that a dimolybdate sugar complex is formed with a pH optimum of 2.5 [199,200]. An early report by Weigel [201] has proposed that molybdenum is present as the oxo-bridged dimolybdate $[Mo_2O_5]^{2+}$ with complexes taking the pyranose form [aldose–O_2–Mo–O–MoO_2–aldose]$^{2+}$. Complexation was observed in the ring-open form with the carbonyl oxygen and hydroxyl oxygens at C2, C3 and C4.

The only X-ray crystal structure known for Mo(VI) and a sugar in a solid state

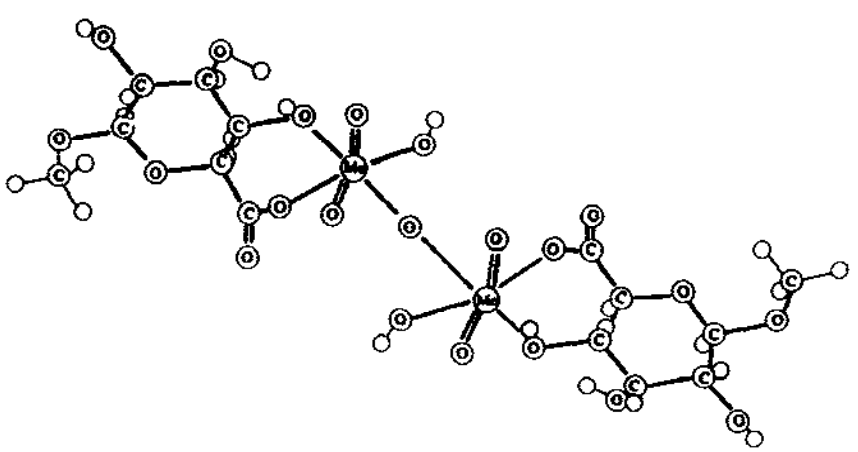
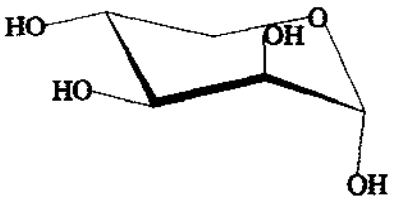


Fig. 9. Schematic diagram of the proposed $[Mo_2O_5(OH)_2(C_7H_{11}O_7)_2]^{2-}$ complex formed between Mo(VI) and MeGlupA (**XXII**). Figure reproduced with permission.

has been reported by Taylor and Waters [202]. They have shown that Mo(VI) complexed with D-lyxose (**XXXV**) occurs through O1, O2 and O3 with a triple-oxygen bridge linking two molybdenum atoms. On the other hand, complex formation between Mo(VI) and sugars in solution has been followed by optical rotatory dispersion [203].

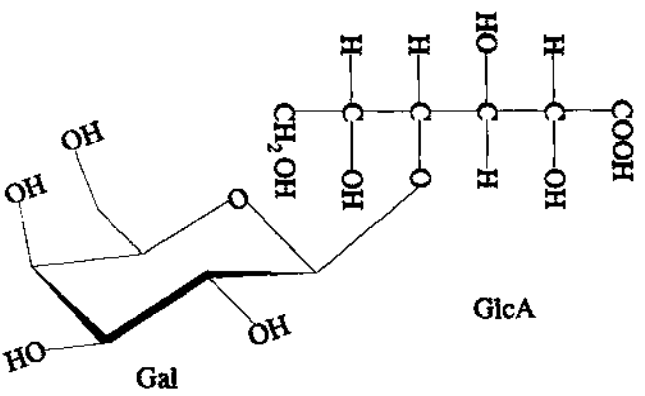


α-D-Lyxopyranose Lyx (**XXXV**)

Similarly, many aspects of the biological function of vanadium are still unknown. Vanadium solution chemistry is also complicated by the formation of oxyanions upon complexation and oligomerization. This property is remarkably dependent on pH and concentration [204]. In order to fully understand oxo-vanadium complexes (mainly in the IV oxidation state), spectroscopic measurements are required. Thus, the most useful techniques used are potentiometry, ESR, CD and UV-Vis spectroscopy.

Since extracellular material is rich in acidic polysaccharides in plants, these polysaccharides allow the roots to remove micronutrients from clay particles (i.e. metal cations) [205]. This prompted some investigations on polygalacturonic acid (cf. **XIV**) [206] which in aqueous solution of $NaVO_3$, V(V) was shown by ESR to form an oxo-vanadium V(IV) species rigidly bound to the polysaccharide matrix through carboxylate groups [53]. Others have investigated the monomer GalpA with oxo-vanadium(IV) [207,208]. Thus, the interaction of GalpA and oxo-vanadium(IV) in solution produces five complex species at different pH. The major species are those involving metal chelation by ionized carboxyl and C4 hydroxyl oxygens above pH 3. From potentiometry, three species were identified as VOL_2H_{-2}, VOL_2H_{-3}, and VOL_2H_{-4}. The VOL_2H_{-2} complex predominates over the pH range 3.5–5.2 as confirmed by ESR. ESR was used to characterize the paramagnetic species as VO^{2+}. However, with GlupA, polymeric species were formed. In addition, studies of complex formation with GalpA in aqueous solution as a function of pH have been shown to reduce V(V) to V(IV) [207,208]. The VO^{2+}, V(IV), GalpA interactions have been studied in detail using ENDOR(ESR) techniques which showed, in combination with potentiometric and other spectroscopic measurements that the binding of the carboxylate and deprotonated sugar hydroxyls (O4 and O3) to the VO^{2+} ion is pH dependent [209]. The disaccharide lactobionic acid (**XXXVI**) has been shown to form similar complexes with the VO^{2+} cation but with higher affinity than the monosaccharide (**XXIII**) [210].

Vanadium has also been studied with simple monosaccharides such as Rib and



D-Lactobionic Acid (XXXVI)

Glc by ^{1}H, ^{13}C and ^{51}V NMR [211]. In these studies, average stability constants were determined with excess of ligand (L:M, 2:1): Rib (230 M^{-1}), Man (24 M^{-1}) and Glc (8.2 M^{-1}). A chiral complex [V(III)Sug_3pyridine$_2$] has been prepared in which the sugar is 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose [212]. It is interesting to note that in complexes with carbohydrates the stereochemistry of Mo(VI) is octahedral and vanadium is trigonal bipyramidal or octahedral. This may be due to the extensive polymerization of molybdate and vanadate, producing oxyanions in solution. It should also be understood that complexation of these metals to sugars depends very much on pH, metal-to-ligand ratio, total metal and ligand concentrations, ionic strength and temperature.

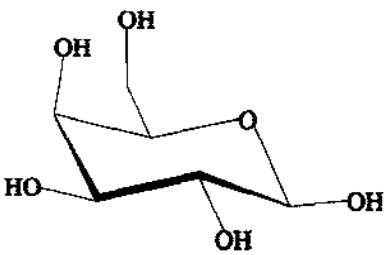
(c) Chromium

Similarly to molybdenum and vanadium, the chemistry of chromium is characterized by the large number of available oxidation states and the formation of oxo-complexes. Some mixed ligand Cr(III) complexes of neutral sugars have been reported [213] but to our knowledge nothing about potential oxo-complexes has been reported. Recently, the gelation of polysaccharides has been studied by monitoring the effect of Cr(III) on the ^{1}H NMR relaxation properties of H_2O. By comparing the NMR results with rheological measurements of the same polysaccharide solutions, kinetic information about gel formation could be determined [214]. Furthermore, dichromate, Cr(VI), has been shown to oxidize reducing GalpA. At low pH, Cr(III) is readily formed via Cr(V) but at pH 5-7, the soluble Cr(V) species is stabilized [215]. It is suspected that the ability of bovine milk to reduce Cr(VI) to Cr(V) is brought about by reducing sugars [216]. The tripeptide glutathione readily reduces Cr(VI) to Cr(III) but in the presence of sugars, a Cr(V) species is stabilized. In this case, the carboxylate does not appear to be involved and the only requirement for complexation appears to be a cis arrangement of vicinal hydroxyls [217]. Cr(VI)

compounds are toxic and carcinogenic; however, it has been proposed that Cr(V) is the actual carcinogenic species [218]. Cr(VI) and Cr(V) carbohydrate chemistry deserves more study in light of these observations.

(vi) Manganese

Manganese is known to form polynuclear complexes with carboxylate ligands [219]. It is therefore not surprising that Mn(II):GlupA complexes have been shown to bind through the carboxylate by the well-established technique [220] of ^{13}C NMR relaxation studies [221,222]. This methodology has also been applied to studies of carbohydrate:lanthanide complexes such as Gd(III) binding to monosaccharides such as NeuNAc [223] and Gal [224]. In glycopeptides, both the oligosaccharide and peptide moieties participate in the Mn(II) binding [225]. The interaction of Mn(II) with two aldopentoses, Rib and Ara, was studied by ^{13}C NMR spectrometry but could not be completely interpreted [226].



ß-D-Galactopyranose Gal (XXXVII)

(vii) Iron

Iron chemistry and biochemistry has been studied extensively and Fe:carbohydrate complexes, such as a complex with Fru, have been described [227]. With monosaccharides and disaccharides after reaction with Fe(III), ESR measurements showed antiferromagnetic behaviour and hence polynuclear complexes [228,229]. In such Fe(III)–carbohydrate complexes, typical Fe–O bond lengths are about 1.95 Å as determined by EXAFS [230]. Furthermore, Fe(III):GlupA and Fe(III):GlcNH_2 complexes have been synthesized and characterized by physio-chemical analysis. The complexes were found to be polymeric in the solid and aqueous state by Mossbauer spectroscopy [231]. An excess of sugar (i.e. hexoses) in the solution prevents Fe(III) hydroxide precipitation and low molecular weight complexes are formed [232].

Since the polygalacturonates of root cell-walls are responsible for about 90% of the root's cation exchange capicity, GalpA:Fe complexes have been studied [233]. ESR and Mossbauer studies of the Fe(II):GalpA complexes show an effect of the degree of hydration, although all forms are octahedral, whereas with Fe(III), as

above, polynuclear complexes are formed [234]. Further studies revealed that the system undergoes redox chemistry leading to the formation of formic acid as well as reduced iron and a Fe:$GalpA_3$ complex [235,236]. Subsequently, the binding constants of GalpA with the ions Cu(II), Fe(II) and Fe(III) were determined (see Table 1). These values suggested an experiment in which a 4:1:5 mixture of Cu(II):Fe(III):GalpA was prepared. This mixture leads to complete reduction of the iron via a Cu(II):GalpA intermediate complex [237]. It is likely that some elements of this system are relevant to the in vivo uptake of Fe(II) in the plant from the Fe(III) in the soil via Fe:GalpA complexes.

Iron absorption in man is not well understood at the biochemical level [238]. It is well established that the primary uptake is of Fe(II) and, for example, Fe(II):GlcA complexes have been used as proprietary medicines for a number of years. A polysaccharide–iron complex named "Nifrex" has been synthesized from $FeCl_3$ and Glc and is currently being used for the treatment of iron deficiency anaemia [239,240]. A poorly characterized product from wine fermentation has been described which is a Fe(II)–carbohydrate complex, that promotes iron uptake [241]. The oligosaccharide is composed of GalpA, Glc, Man and Xyl with an estimated molecular weight of around 1500 Daltons [242]. Iron binding was localized to a GalpA-Man disaccharide but this disaccharide was not able to promote iron uptake [243]. Further investigation of Fe(II)–carbohydrate complexes and related Fe(III) reduction chemistry for the preparation of assimilable iron by plants and animals is warranted.

(viii) Cobalt

Some optically active mixed ligand complexes with Co(III) and sugars have been formed, for example $[Co(NH_3)_4(Rib)]_2(SO_4)_3 \cdot 4H_2O$ [244]. Similar octahedral mixed complexes with GlupA or GlcA and Co(III) have been reported [245]. Co(II), Ni(II) and Cu(II) binding to $GlcNH_2$ and its methyl glycoside were investigated by potentiometry (see Table 1) [48]. At high pH, stable deprotonated sugar hydroxyls were detected, although it is not strictly possible to rule out OH^- ion-containing species without hydroxyl deprotonation from potentiometry alone (cf. Fig. 17, below).

Cobalt(II) binds to the glycosylaminoglycan chondroitin sulfate such that 20% of the Co(II) is site bound and 30% territorially bound. Site binding was determined by ^1H NMR and total binding from self-diffusion coefficient measurements [246].

(ix) Nickel

Nickel binding to carbohydrates has only been studied in the last few years. For example, Kozlowski et al. have reported Ni(II) binding to $GlcNH_2$ [247]. However, Makridou et al. could not detect a complex with GlupA or GalpA by potentiometry, although they did find complexes with Eu(III), Pb(II), Cu(II) and Cd(II) (see Table 1) [248]. We have shown that Ni(II) does indeed interact with the

carboxylate of the β-methyl glycoside of GlupA (**XXII**) as shown in Fig. 10. In these experiments, the ^{1}H NMR T_1 values of (**XXII**) were measured by the inversion recovery technique in the presence and absence of Ni(II). The lower spectrum in Fig. 10 shows all the resonances simultaneously inverted and hence similar T_1 values in the absence of metal. In the upper trace, the H5 (adjacent to the carboxylate) resonance is seen to revert, i.e. much shorter T_1, while the other resonances are still inverted. These data demonstrate that the carboxylate is the binding site for Ni(II) [61].

This study was initiated after studies of Ni(II) metabolism in humans. Ni(II) is known to be mostly transported, complexed to albumin, in blood [249]. It is also known that, after oral administration of Ni(II), most Ni(II) is rapidly excreted in urine. This prompted the question of how Ni(II) bound to albumin is exchanged so that it can pass through the kidney cells into the urine. Subsequently, two Ni(II)-binding fraction were found in kidneys. One fraction is an acidic peptide and the other is acidic oligosaccharides [250,251]. Subsequent characterization of this pool shows it to contain the monosaccharide residues $GlcNH_2$, GlupA and L-iduronic acid (IdopA, **XXXVIII**), and sulfate [252]. These are the components of heparin and heparan sulfate, a polymer which is less sulfated and contains less IdopA than heparin. Heparan sulfates are components of kidney basement membranes [253]. Their metal binding properties have been studied using radioisotopes and an ultracentrifugation assay. Although most of the binding isotherms could be modelled using the Manning theory, it was concluded that some specific sites exist for Ni(II), Mn(II) and Ca(II) with a $K_a = 4.5 \times 10^6$ M^{-1} [254,255]. Figure 11 shows a binding isotherm that demonstrates specificity for Ni(II). These Ni(II)-carbohydrate complexes must have a role in Ni(II) metabolism in human kidneys. Indeed, we have shown that these oligosaccharides are found inside kidney cells and that their concentrations are sensitive to conditions that affect heparan sulfate turnover. These biochemical observations, along with the chemical observation that acidic sugars bind metals at low pH and higher salt concentrations than most metal binding peptides, has led to the hypothesis that these sugars act as metal scavengers in the low-pH high-salt environment of lysozymes [5].

(x) Copper

After Ca(II) binding, Cu(II) binding to carbohydrates has been the most studied. This is in part due to several observations of biologically significant interactions between Cu(II) and sugars. For example, an exopolysaccharide fraction from the pathogenic bacteria *Pseudomonas aeruginosa* has been shown to form a Cu(II) complex which has spectral characteristics similar to a Cu(II):GlupA complex [256]. Other observations include an effect of Fru on the uptake of Cu(II) in rats [257] and the demonstration of a cooperative effect between Cu(II) and heparin and human fibrinogen [258], and other proteins [259].

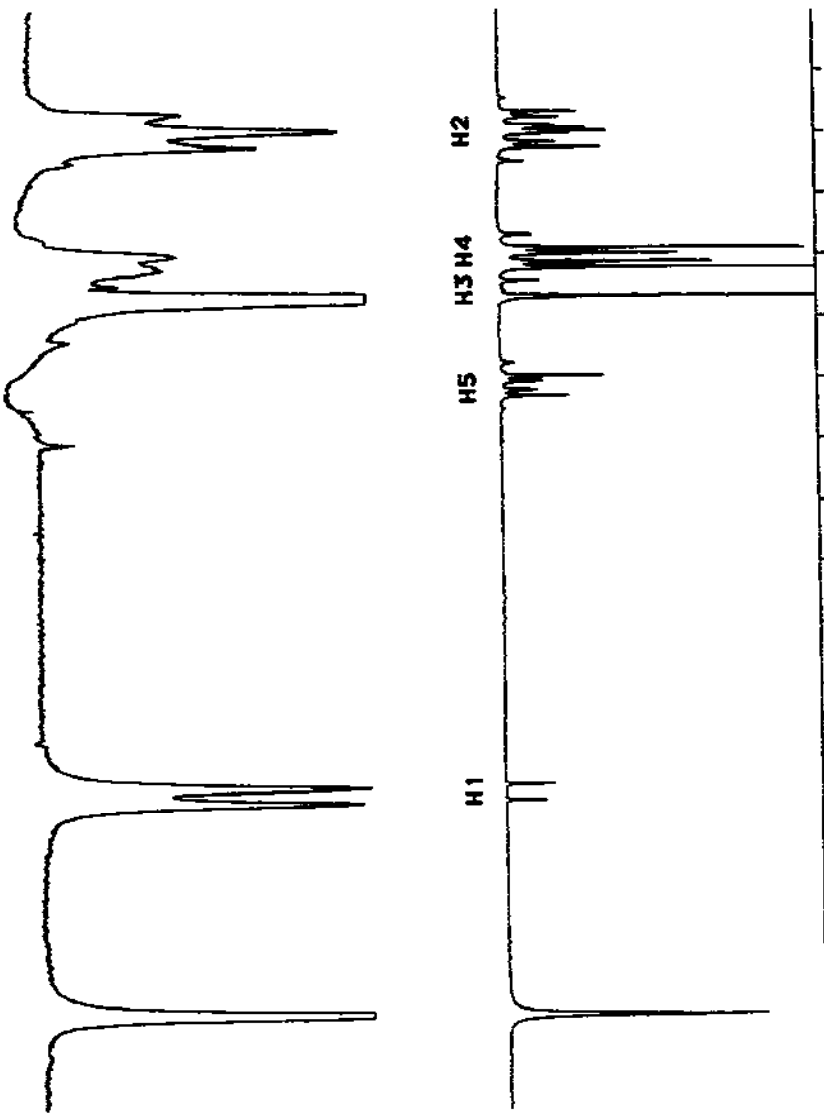


Fig. 10. ^{1}H NMR inversion recovery experiment of Na salt of MeGlupA (**XXII**) in the presence of (top) and absence (bottom) of 0.5% $NiSO_4$. Figure reproduced with permission.

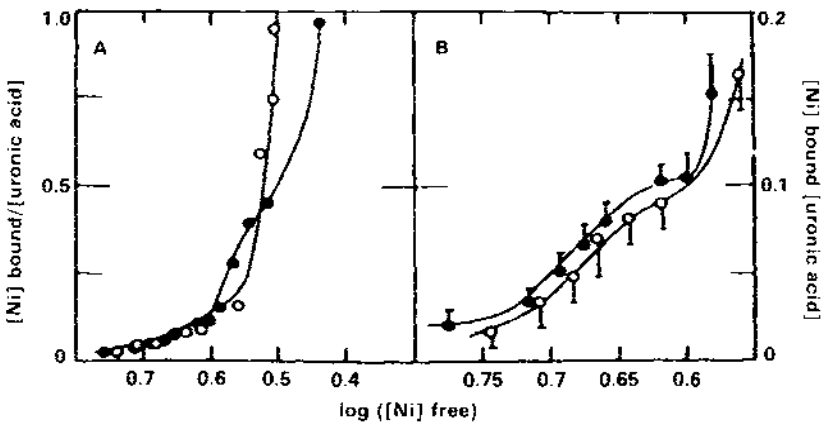


Fig. 11. Binding of ^{63}Ni(II) to bovine glomerular basement membrane. Plots of [bound Ni(II)] versus log [free Ni(II)] following Klotz [352]. Panel B shows in detail expansion of the lower binding region of panel A. (○) Binding in Tris–HCl buffer, 5 mM pH 7.4; (●) in Tris buffer with the addition of 140 mM NaCl. Values are means ±S.D. of bound Ni(II) values from triplicate assays on the same batch of glomerular basement membrane. Figure reproduced with permission.

Yet another impetus for studying Cu(II)-carbohydrate complexes, is the suggestion that Cu(II) based drugs may show efficacy in the treatment of rheumatoid arthritis [260,261]. This has led to the demonstration of Cu(II) binding to hyaluronate, a constituent of synovial fluid. Using a spectrophotometric method, a binding constant of 3×10^3 mol^{-1} was determined from a unique band at 238 nm [262]. CD studies demonstrated that this absorption is a charge-transfer ligand to Cu(II) band. These CD studies also suggest a Cu(II)-induced conformational change of the hyaluronate at neutral pH [263]. ^{1}H and ^{13}C NMR relaxation measurements suggest binding to the carboxylate and O1 of the GlupA residues of hyaluronate [264]. A polarographic study confirmed complex formation [265]. This Cu(II)-hyaluronate complex has been proposed to lead to Cu(II) to Cu(I) reductions followed by the formation of free radicals and subsequent degradation of the hyaluronate and hence typical arthritic symptoms.

Other groups have studied Cu(II) binding to monosaccharides [266] by spectroscopic methods. The most comprehensive study is that of Weigel and co-workers [267] who have studied TLC using metal impregnated silica gel and other supports. They have tabulated binding to over 30 oligosaccharides with Cu(II) and Ca(II) exchanged silica gel. All sugars tested, interacted with the Cu(II) exchanged silica gel. Acidic and amino sugars were essentially 100% complexed and even some polyols, such as mannitol, were significantly complexed. They also tested Na(I), Mg(II), Al(III), Ca(II), Cr(III), Fe(III), Ni(II), Zn(II), Sr(II), Cd(II) and Ba(II) but all were less effective than Cu(II). This work has led to the development of preparatively useful separations based on these supports. Also, Cu(II) binding to aminosugars have been investigated by potentiometry, polarography and ESR spectroscopy

[48,268,269]. In particular, $GlcNH_2$, with Cu(II) forms $[CuL_2]$ and $[CuH_{-2}L_2]$, where L is the $GlcNH_2$ ligand in the pH region 6–9. No complex was observed with Cu(II):GlcNAc.

Kohn and Hirsch have investigated Cu(II) binding to GalpA and to some derivatives as shown in Fig. 12 [270]. Their results are expressed as the degree of association ($\beta = 1$ corresponds to 100% complex). These data can be interpreted to show the importance of O4 in binding as the 4-deoxy compound (**XXIVa** in Fig. 12) was a poorer ligand. Angyal has questioned this result and favours binding to O5 and the carboxylate [41]. Kohn has done similar studies examining Ca(II), Sr(II), Cd(II), Zn(II), Pb(II) and Cu(II) binding to the oligomers (DP 1–6) of GalpA (from **XII**) [271]. Figure 13 shows some of these results, which demonstrate that Ca(II), Sr(II) and Zn(II) are only electrostatically bound whereas Cd(II), Pb(II) and Cu(II) form chelates. Note that binding to Cu(II) essentially levels off at DP = 3. These conclusions are in accord with the ESR and IR studies of Deiana et al. which show that Cu(II) and VO(IV) form inner-sphere carboxylate complexes, whereas Mn(II),

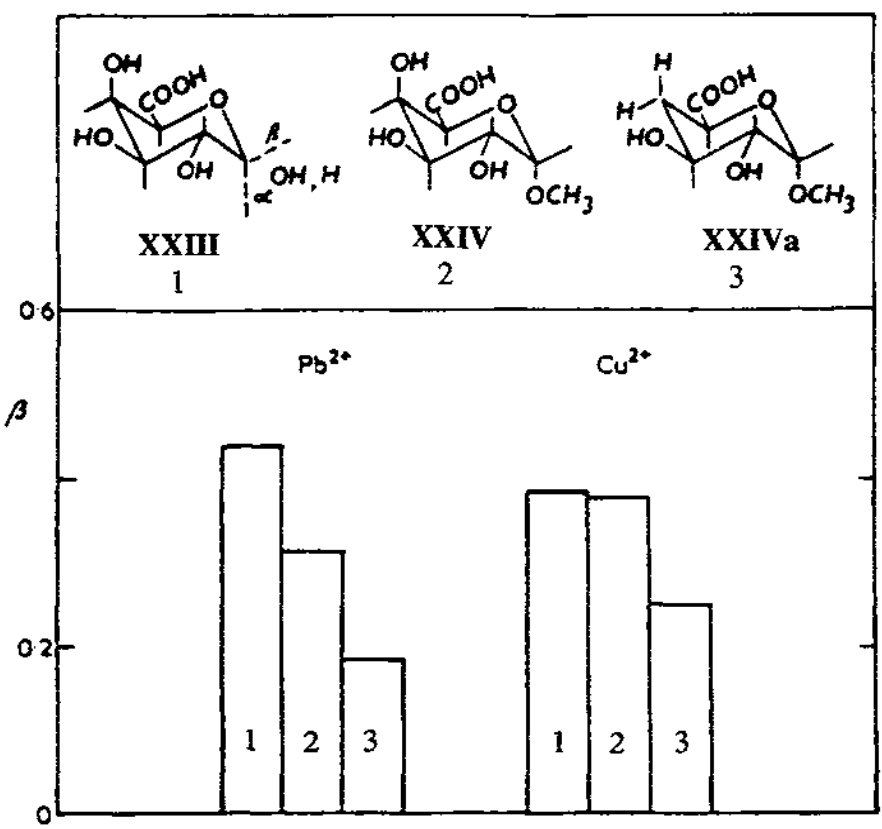


Fig. 12. Binding of Pb(II) and Cu(II) to GalpA (**XXIII**) and its derivatives **XXIV** and **XXIVa**. β is the degree of association. Figure reproduced with permission.

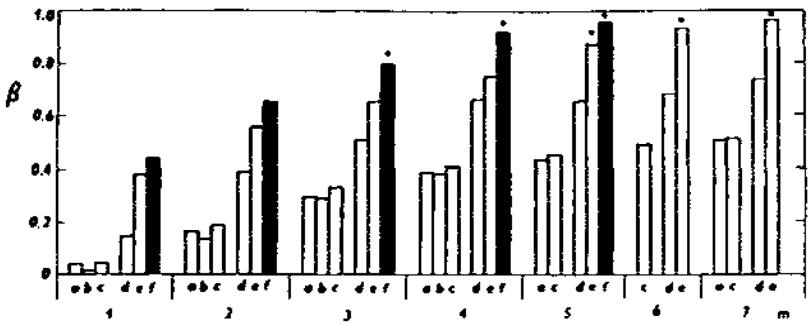


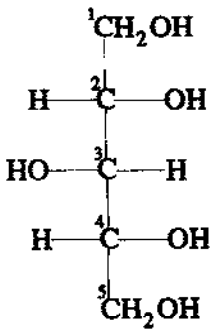
Fig. 13. Degree of association, β, of divalent cations with oligo-D-galactosyluronates of various values of DP (m). Key: asterisk denotes system with partial precipitation of oligomers; electrostatic bond, a, Ca(II); b, Sr(II); c, Zn(II); formation of chelates; d, Cd(II); e, Cu(II); and f, Pb(II). Figure reproduced with permission.

Ni(II) and Co(II) form outer-sphere complexes with GalpA [272]. In addition, the results of ESR and potentiometric studies on the complexation of Cu(II) and GalpA have been reported. The Cu(II):GalpA formation constants were determined to be: $\log K_1 = 2.16$ and $\log K_2 = 2.05$. Comparison of Cu(II):GalpA complexes derived from pectins showed that weaker bonds were formed with Cu(II) in galacturonan polymers than that of the GalpA monomer [273].

In other studies, Cook et al. [274] have proposed a bidentate coordination site involving O3 and the carboxylate oxygen (O6) at pH 4.5 for a Cu(II):GlupA complex with the sugar inverted to the high energy $^1C_4$ conformation. This mechanism for complex formation is based on the ability of Cu(II) to catalyze mutarotation. The energetically preferred conformation for the Cu(II):GlupA complexes are the normal $^4C_1$ conformations of D-pyranose sugars. A number of other investigations on the interaction of Cu(II) with GlupA have been made. These include studies using potentiometry [248], colorimetry [275] and poloragraphy [276,277]. Two models have been proposed. Makridou et al. [248] proposed that Cu(II) coordinates to the carboxylate group only. Aruga [275], on the other hand, postulated that, at pH 4.3, Cu(II) was bound in a bidentate manner directly through the carboxylate group and the endocyclic oxygen O5 on the ring. All reports concur on the carboxylate as a binding site but the roles of other sugar oxygens in binding have been interpreted differently.

Recently, Angyal has re-examined Cu(II) binding to polyols and suggests that, above pH 5, complex formation is attributed to the $[Cu_2(OH)_2]^{2+}$ ion or similar binuclear ions. The formation of tetradendate binuclear complexes was postulated with compounds having four hydroxyl groups in suitable steric relationships, such as xylitol **(XXXIX)** [278].

Copper(II) binding to chondroitin sulfates has been studied by activity measurements using potentiometry, viscometry, ESR and NMR spectroscopy [279]. Together these studies show that Cu(II) binds to the carboxylate, but only has electrostatic



Alditol D-Xylitol (XXXIX)

interactions with the sulfates and no interactions with the amides. Another group emphasizes the need for using several techniques [280] to study binding and they have developed a polarographic method to quantitate binding [281].

Copper(II) binding to heparin has also been studied and a similar charge transfer band at 237 nm as for Cu(II)-hyaluronate, was used to determine a binding constant of 1×10^4 M^{-1} [282]. Parallel CD studies showed a Cu(II)-induced conformational change. Equilibrium dialysis also supports cooperative binding [283]. Changes of the IdopA ring conformation after Cu(II) binding are the most likely source of these overall conformational changes. It should be noted that the oligosaccharide fraction from kidneys also binds Cu(II). Recently, Perlin and co-workers have shown that Cu(II) binds to heparin at levels less than 10^{-3} mol of Cu(II) per dimeric unit of polymer, as evidenced by paramagnetic relaxation effects on the NMR spectra of the polysaccharide. N- or O-desulfation abolished this specific binding and no other glycosylaminoglycans tested showed this effect. Similar relaxation experiments with Gd(III) demonstrated a wider range of binding specificities [284]. These studies have been extended to show specific binding of Fe(III) too [285].

The IR of partially hydrated films and aqueous solutions of various heparins in the presence of various cations demonstrates that, in addition to the simple electrostatic interactions, specific cation effects and the hydration pattern of the polysaccharide must be considered [286]. Cu(II) and Mg(II) have the most pronounced effects. Analysis of the spectra revealed an interesting dependence of the carboxylate stretching frequency on the cation polarizing power, as shown in Fig. 14 [287]. Obviously, Cu(II)–carbohydrate complexes have a role in biological processes.

(xi) Zinc

Zinc is commonly found bound to proteins in nature, either as a part of a catalytic site in enzymes [288] or as a structural component, as in DNA binding "zinc fingers" [289]. Complexes of Zn(II) with the monosaccharides Fru [290] and

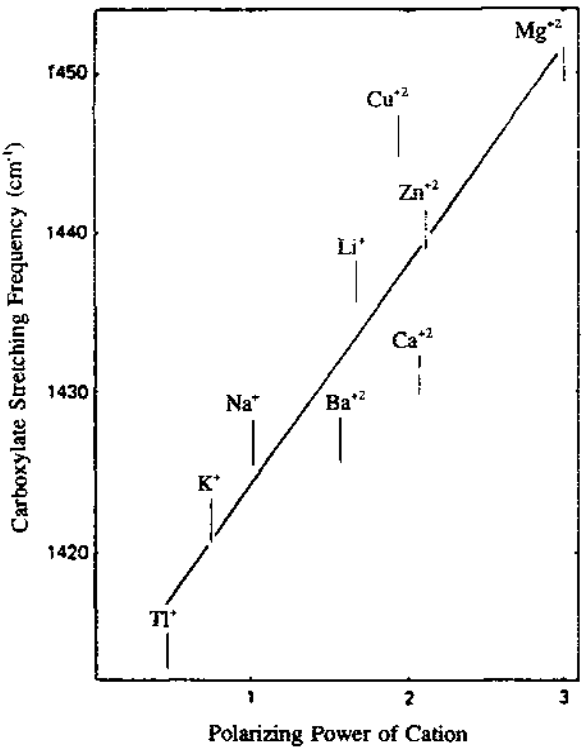


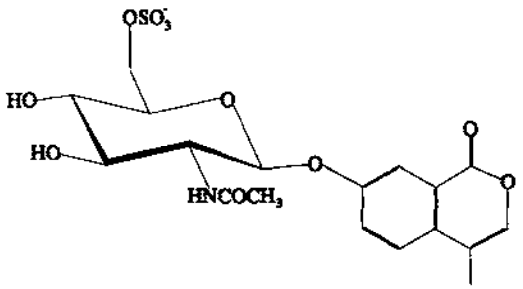
Fig. 14. Variation of carboxylate stretching frequency with cation polarizing power. Polarizing powers were calculated from the equation [353]

Polarizing power = $(z/r)(5z^{1.27}r^{0.5}/I)$

where z is ionic charge, r is ionic radius in A, and I is ionization potential in volts; these data were obtained from ref. 354. Figure reproduced with permission.

GlupA [291] have been described, as has electrostatic binding to oligogalacturonates [41] (see Fig. 13). The most important result is that heparin binds Zn(II) better than other glycosylaminoglycans do [292–295].

Since we have identified a Ni(II) binding oligosaccharide pool in kidneys which is likely a heparan sulfate degradation product [5] and in order to ascertain which residues in heparin may be responsible for its Zn(II) binding capacities, we have investigated metal binding by ^{1}H and ^{13}C NMR to some monosaccharide derivatives [61]: namely 4-methylumbelliferyl-2-deoxy-2-acetamido-6-*O*-sulfo-β-D-glycopyranoside (GlcNAc6S, **XL**), methyl-β-D-glycopyranosiduronic acid (MeGlupA, **XXII**) and methy-α-L-idopyranosiduronic acid (MeIdopA, **XLI**). The diamagnetic Zn(II) ion and the octahedral paramagnetic Ni(II) ion were used as probes. GlcNAc6S (**XL**) was used as a model for O-sulfates. Only weak interactions with the sulfate group were found from T_1 measurements in the presence of Ni(II). The $^{4}C_1$ ring conformation of MeGlupA was not perturbed by binding to its carboxylate (see Fig. 10) and little evidence exists for chelation.



4-Methylumbelliferyl-2-deoxy-2-acetamido-6-O-sulfo-ß-D-glucopyranoside GlcNAc6S

(XL)

By contrast, the ring conformation of the sodium salt of MeIdopA is affected by the addition of Zn(II) > Pb(II) > Cd(II) > Ca(II) ≫ K(I) ions at the same approximate mole ratio. This binding manifests itself by ^{1}H and ^{13}C chemical shifts, and ^{1}H coupling constant changes. The chemical shift changes are summarized in Figs. 15 and 16, at the same approximate mole ratios. In Figs. 15 and 16, zero chemical shift

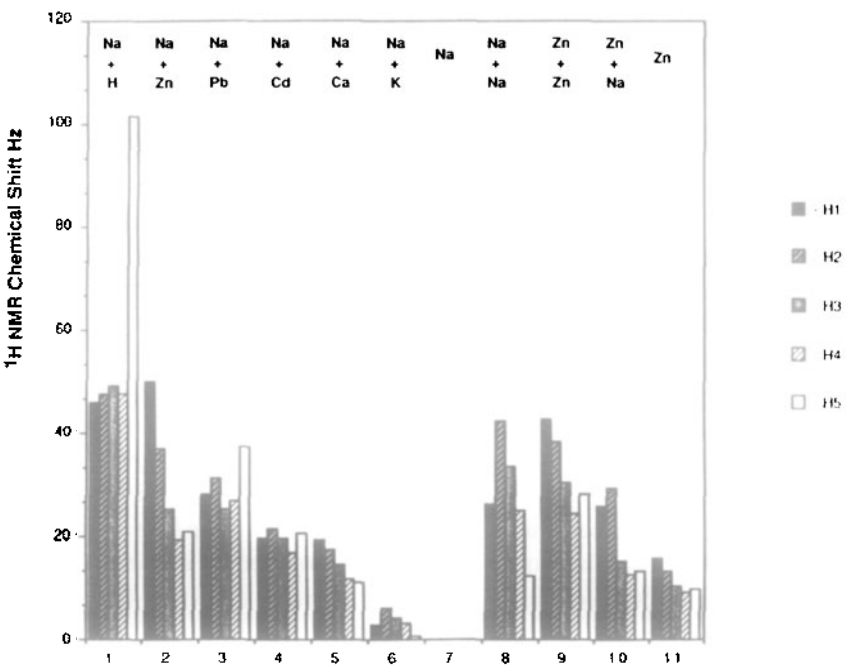


Fig. 15. ^{1}H NMR chemical shifts of MeIdopA (**XLI**) and its Na(I) or Zn(II) salts in the presence of added metal cations. Entries 1–11 are MeIdopA, NaMeIdopA + 1.8 eq. $ZnCl_2$, NaMeIdopA + 1.3 eq. $Pb(OAc)_2$, NaMeIdopA + 1.3 eq. $CdCl_2$, NaMeIdopA + 1.9 eq. $CaCl_2$, NaMeIdopA + 1.8 eq. KCl, NaMeIdopA, NaMeIdopA + 36 eq. NaOAc, ZnMeIdopA + 8.6 eq. $Zn(OAc)_2$, ZnMeIdopA, ZnMeIdopA + 5.6 eq. NaOAc. Figure reproduced with permission.

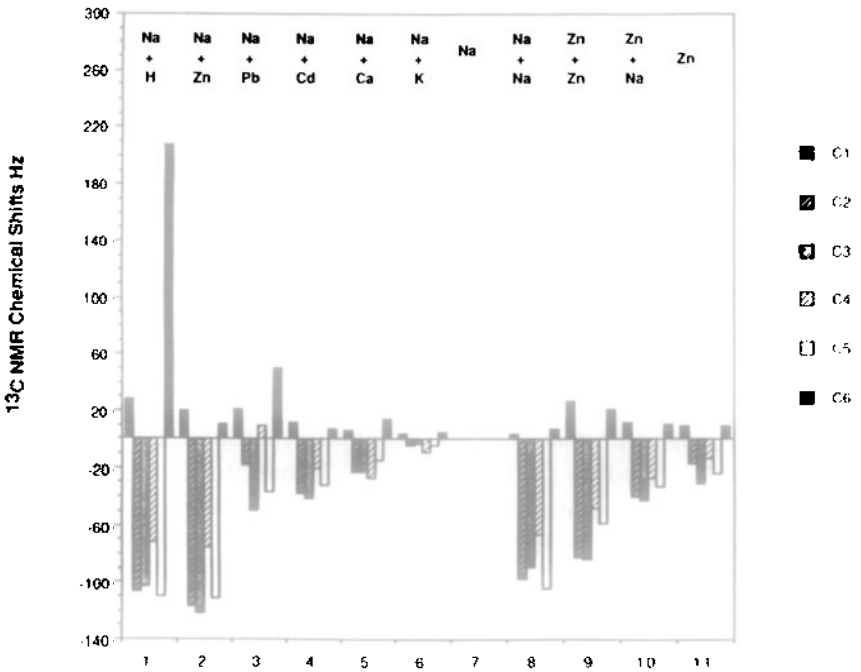


Fig. 16. ^{13}C NMR chemical shifts of MeIdopA (**XLI**) and its Na(I) or Zn(II) salts in the presence of added metal cations. Entries 1–11 are the same as in Fig. 15. Figure reproduced with permission.

is arbitrarily assigned to the Na(I) salt and metal induced shifts are positive downfield in Hz.

Metal binding to ligands can induce chemical shifts by at least two mechanisms: namely direct metal–ligand electronic effects or by induced ligand conformational changes. It is usually difficult to separate these two mechanisms. In this case, the pronounced chemical shifts are for carbons and protons not likely to be directly involved in metal coordination and thus can be attributed to induced conformational changes (see Figs. 15 and 16). This hypothesis is substantiated by comparison with the small metal-induced shifts of the C5 epimer MeGlupA (not shown). Also the ^{13}C resonance of the carboxylate of MeIdopA was not observable in the presence of 0.5 mol% $NiSO_4$, strongly suggesting coordination to the carboxylate [296].

An apparent 1:1 binding constant for $Zn(OAc)_2$ and MeIdopA in D_2O was found to be 154 M^{-1}. This equilibrium constant was determined by curve fitting the NMR data by iterating for the equilibrium constant and the chemical shift of the 1:1 complex. Corrections for Zn(II) binding to the acetate counter ion were made [297]. The shifts for MeGlupA (not shown) were too small to make such a determination reliable.

Figure 17 shows the results of some acid–base potentiometric titrations of MeGlupA in the presence of increasing amounts of $ZnCl_2$ [298]. The pH changes in the range 2–7 were too small to calculate a binding constant. Above pH 7, a

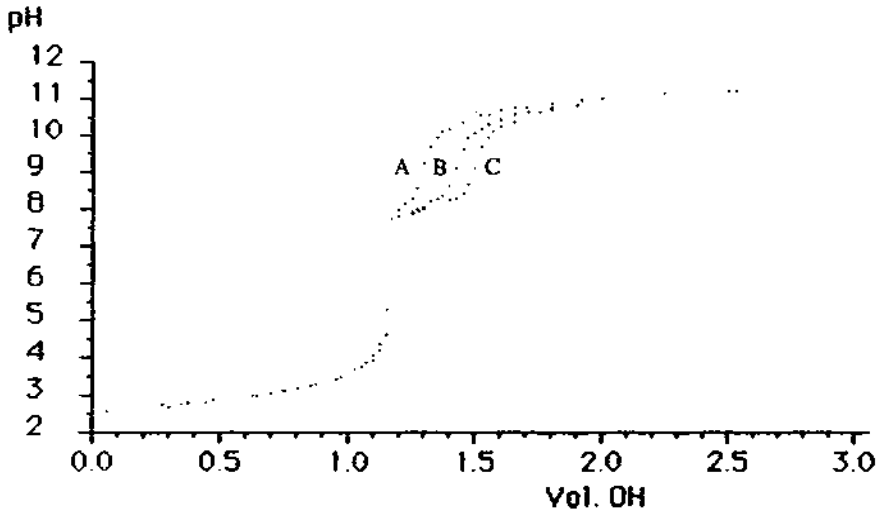


Fig. 17. Potentiometric titrations of 0.603 mM MeGlupA (**XXII**) in the presence of 0.38 mM $ZnCl_2$ (A), 0.41 mM $ZnCl_2$ (B) and 0.43 mM $ZnCl_2$ (C). $I = 0.15$ (KCl) and $T = 25$ °C. The pH was monitored using a glass electrode and a pH meter. Aliquots of 0.103 M NaOH were added using a Radiometer automatic burette, initial volume 35 ml. The endpoints between pH 7.5 and 10 indicate 2 protons displaced per Zn(II) ion.

marked [Zn(II)]-dependent endpoint is observed that corresponds to two protons per Zn(II). Zn(II) at high pH precipitates due to the formation of polynuclear Zn(II) hydroxides [299]. Since no precipitation was observed at the end of the titration and NMR studies of MeGlupA and Zn(II) at pH values >10 showed no significant perturbations from spectra at pH 5 (not shown), it is presumed that a MeGlupA:Zn:$(OH)_2$ complex is formed. Specifically, the sugar hydroxyls are not deprotonated. In those cases where excess acetate ions are present, it is likely that they compete to fill the Zn(II) coordination sphere.

As deduced from the ^1H coupling constants and NOE experiments, the Na salt of MeIdopA is suggested to be an equilibrium mixture of the unusual ^2S_O and the usual $^1C_4$ ring conformations (for a discussion of IdopA ring conformations, see refs. 300 and 301). Cation coordination to the carboxylate group shifts this equilibrium towards the $^1C_4$ conformation and suggests additional binding to O5. This effect appears to be electrostatic in nature as excess Na(I) and protonation produce similar shifts. Huckerby and co-workers have shown that added monovalent ions had little effect on the NMR spectra of some heparin-derived oligosaccharides [302] and Van Boeckel et al. [303] have reported that 3M NaCl stabilized the $^1C_4$ ring conformation of the IdopA2S residues in different heparin-derived oligosaccharides. Similar results were found for the addition of Ca(II) to IdopA-containing oligosaccharides [304]. These results are in accord with our results.

Zinc(II) binding is probably electrostatic, resulting in a reduction of electron density on the carbohydrate oxygens. Such a diminution in electron density would

reduce the repulsive interactions in the $^1C_4$ conformation, notably the 1,3-diaxial interactions between O1 and O3, and between O2 and O4. This result, although speculative, leads us to propose a model where the metal ions are bound to the carboxylate and to O5 (see Fig. 18). Molecular modelling experiments with Zn(II) and MeIdopA suggest that this arrangement can be accommodated while maintaining 2 Å bond lengths to both oxygens [305]. Such an arrangement requires that the O6C6–C5O5 dihedral angle is near 0°. This value is indeed found in several X-ray crystal structures of uronic acid salts [306–309]. All of these compounds exhibit chelation to their counter-cations via the carboxylate oxygen and O5. Alternate chelation to O4 is less likely due to these observations. These speculations are in accord with the observations of Angyal concerning metal binding to GalpA [39].

Lead(II) complexation is different from the other ions and suggests some covalent character. The chemical shift patterns are clearly different, notably the ^{13}C shift of the carboxylate (see Figs. 15 and 16). Since MeIdopA likely provides only two binding sites, its coordination chemistry will be satisfied by cations that can fit these sites simultaneously. Thus tetrahedral geometries such as Zn(II) and Cd(II), and octahedral geometries such as Ni(II) can be accommodated. Ions that are either too small or too large, or only weakly complexing, will not bind well. In order to get stronger and more specific sites, flexible oligosaccharides which can fold to form additional sites are necessary. Polymeric substances are also not suitable because binding to polymeric species is a different phenomenon for at least two reasons. One is the ion cloud effect of polyelectrolytes and the second is the restriction in conforma-

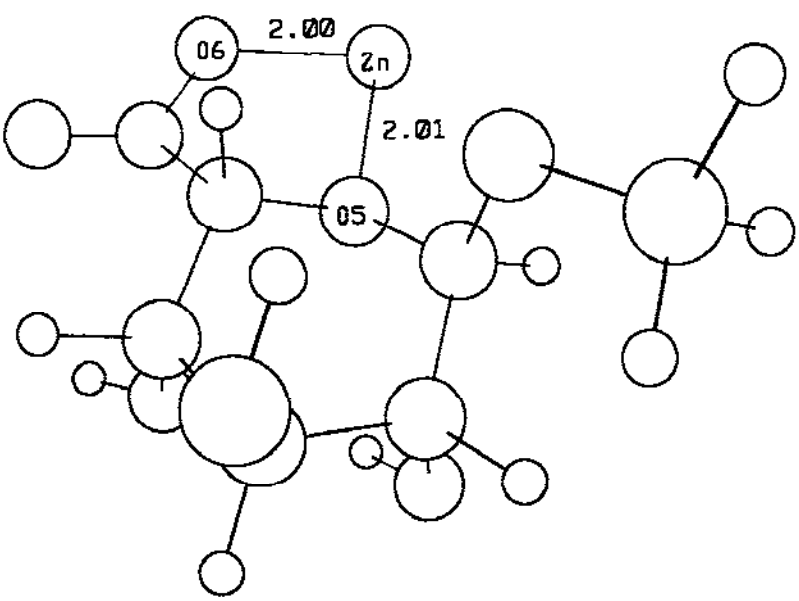
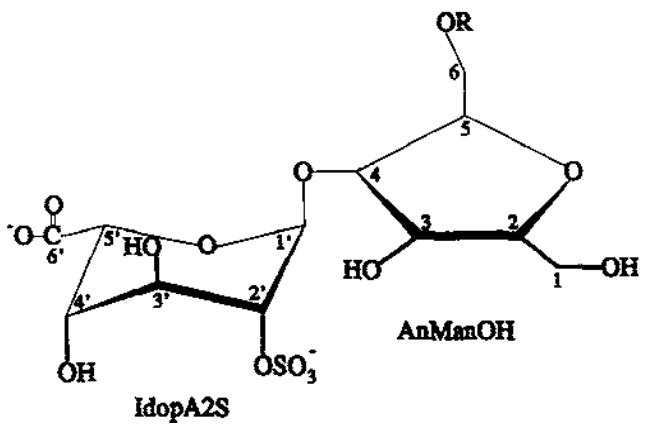


Fig. 18. Ball and stick representation of the complex between Zn(II) and MeIdopA (XLI).

tional space imposed by the secondary structure of the polymer. Thus, di- or larger oligosaccharides with the reducing end fixed as glycosides are required.

On this basis we have initiated a study of heavy metal binding to heparin-derived disaccharides such as disaccharides: 2-*O*-sulfo-4-*O*-(α-L-idopyranosyluronic acid)-2,5-anhydro-D-mannitol, disodium salt (**XLIIa**) and 2-*O*-sulfo-4-*O*-(α-L-idopyransyluronic acid)-6-*O*-sulfo-2,5-anhydro-D-mannitol, trisodium salt (**XLIIb**). For disaccharides (**XLIIa**) and (**XLIIb**), weak dative [310] bonds between the hydroxymethylene O6s of the hydroxyl or sulfate groups at C6 of the AnManOH residues were also postulated by comparing NOE and T_1 relaxation measurements with calculated NMR observables. These results suggested that suitable derivatives of iduronic acid could exhibit chelation [311].

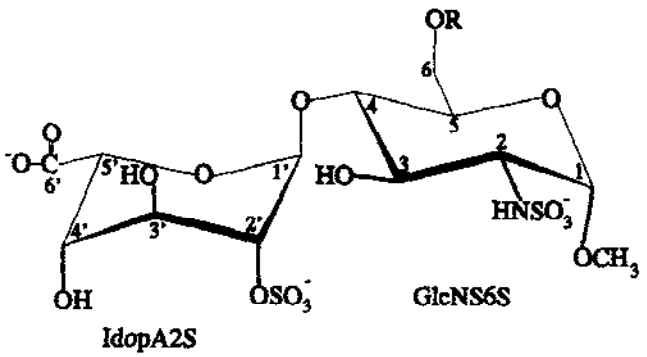


Disaccharides Derived from Heparin by Nitrous Acid Hydrolysis and $NaBH_4$ Reduction

R = H is (XLIIa) and R = SO_3 is (XLIIb)

Subsequent studies with the disaccharides: methyl, 2-*O*-sulfo-4-*O*-(α-L-idopyranosyluronic acid)-2-deoxy-2-sulfamido-α-D-glucose, trisodium salt (**XLIIIa**) and 2-*O*-sulfo-4-*O*-(α-L-idopyranosyluronic acid)-2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucose, tetrasodium salt (**XLIIIb**). These two disaccharides were shown to bind tracer amounts of $^{63}Ni(II)$ and $^{67}Cu(II)$ using chromotographic assays in close similarity to the results obtained with the oligosaccharides from human kidneys. Subsequently, ^1H NMR complexation studies of (**XLIIIa**) and (**XLIIIb**) with $Zn(OAc)_2$ suggested chelation (complexation constants are over a 100 times larger than for MeIdopA, **XLI** (see Table 1)). A conformational analysis of the metal free and metal bound solutions was made by comparing calculated NOEs, T_1s and Js with experimental values. The results suggest metal-binding conformations with the carboxylate and ring oxygen of the IdopA2S residues ($^1C_4$ conformation) and either O3 of the GlcNS(6S) residues or the sulfate oxygens of the 6-sulfate for providing additional

chelating sites. A representation of the proposed Zn(II) chelate is shown in Fig. 19 [312].



Synthetic Disaccharides Related to Heparin R = H is **(XLIIIa)** and R = SO_3 is **(XLIIIb)**

(xii) Lead

Lead exposure is a topical environmental problem affecting, in some way, most of the population [313]. It is therefore of some interest to develop water-soluble, non-toxic Pb(II) chelating agents to augment or replace the existing ones. Carbohy-

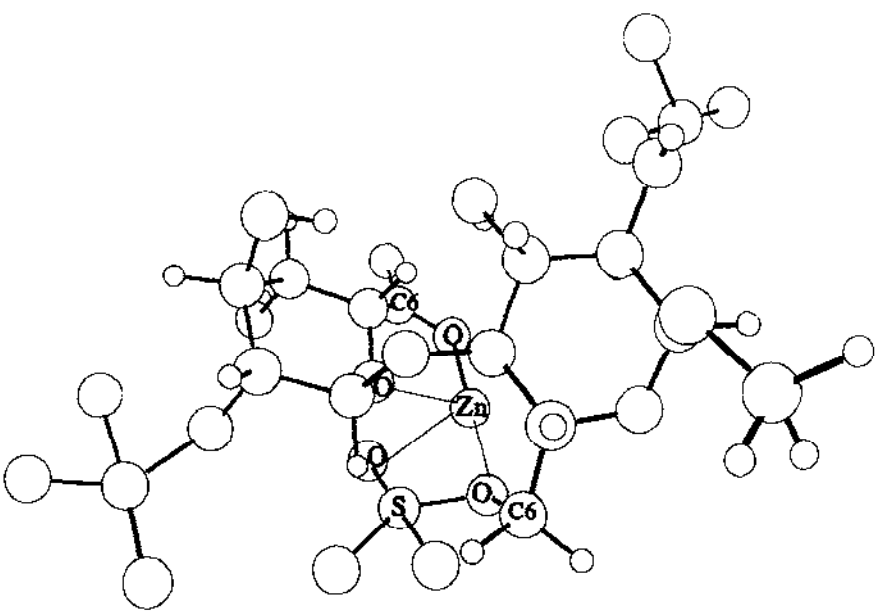


Fig. 19. Ball and stick representation of the complex between Zn(II) and disaccharide **(XLIIIb)** showing chelation by the *O*-sulfate.

drates are both water soluble and generally non-toxic and therefore good candidate substances. This motivation was behind the work of Kohn, who showed that Pb(II) interacted strongly with GalpA oligomers [314] (see Fig. 13). The enthalpies of interaction of the methyl glycoside of Rib with Pb(II) have been measured and are greater than those for Ca(II) or La(III) but are still small [315]. However, this does indicate the affinity of Pb(II) for carbohydrates. This greater affinity for Pb(II) than Ca(II) has been quanititated by potentiometry using Pb(II) ion specific electrodes and studying some pentoses [316].

It has also been shown that GlcA and GlupA interact with Pb(II) [317,318]. In fact, Pb(II) is known to be among the best cations for inducing gelling of polysaccharides [319]. A study using CD and UV spectroscopy by Paoletti and co-workers demonstrates a strong interaction via the carboxylates of GlupA-containing polymers [320]. A crystal structure of Pb(II):GlcA also shows coordination via the carboxylates of four GlcA monomers (see Fig. 20). Two of the GlcA monomers also chelate via O2 to give a quasi-octahedral coordination sphere with Pb–O bond lengths from 2.43 to 2.71 Å [321]. These bond lengths are markedly longer than the Zn–O bond lengths and in part may rationalize the different binding of Pb(II) to MeldopA. Given the importance of Pb(II), it seems worthwhile to investigate Pb(II)–carbohydrate complexes further.

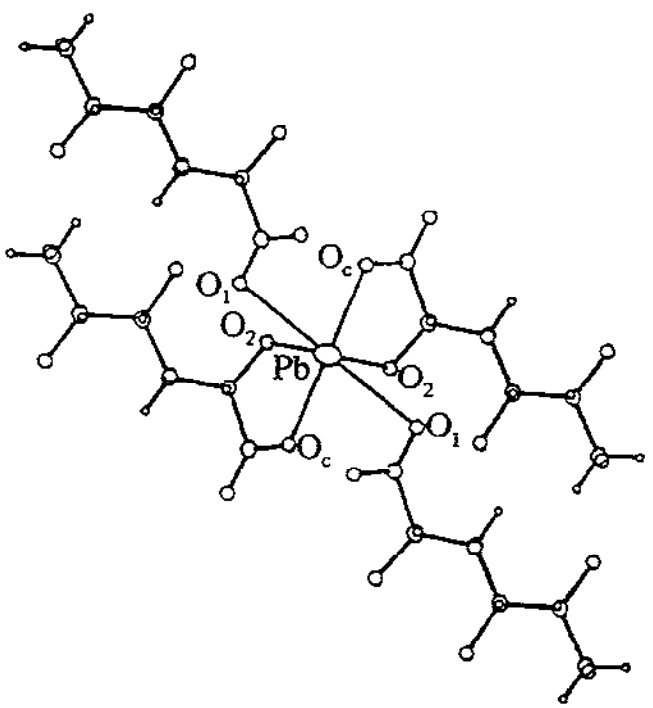


Fig. 20. Projection along the twofold axis of the six-coordinated polyhedron around Pb(II). Figure reproduced with permission.

(xiii) Others

The oxo-cation UO^{2+} coordinates with several saccharides such as GlupA [322], Fru [323] and Ara [324] as demonstrated by IR spectroscopy. A complex between Ag(I) and GlupA and its lactone [325,326] has been described by FT-IR spectroscopy. A novel arseno sugar with a C—As bond, has been isolated from brown algae [327]. As candidate substances with biocidal activity, a series of Group IVA (Si, Sn and Pb)-substituted Gal derivatives have been prepared [328]. Solution complexes between GlupA and Nb(V) have also been prepared [329].

D APPLICATIONS

(i) Biotechnology

In recent years, the number of publications on extracellular polysaccharides has increased dramatically, mainly due to their attractive commercial and technological exploitations. The extracellular polysaccharides show extraordinary complexing ability to cations. There is potential to use these extracellular polysaccharides for the extraction of metals which are economically important. Several extracellular polysaccharides have been isolated that have commercial application as gelling or thickening agents [330–332]. Although xanthan gum is the only commercially significant microbial polysaccharide to be used in food, industrial and oil field applications, gellan gum, a microbial polysaccharide is now sold for use as a gelling agent to replace agar.

Most alginates have gelling properties with metallic ions. Alginates are typically found in brown seaweed (e.g. *Phaeophyceae*) [333]. Other seaweed polysaccharides such as the carrageenans from species of *Rhodophyceae* also produce gelling properties with different univalent cations [334]. Thus, these types of extracellular polysaccharides have many possible commercial uses, e.g., as food additives, gums, coating materials and flocculants.

Furthermore, chitin derivitives and particularly chitosan (-(β1,4)GlcNH$_2$–, **VIII**) are employed for the selective removal of transitition metals from brines. Some work aimed at purifying water from metals [335] has appeared which utilizes derivitized chitin as the ion chelator [336,337]. These materials are effective for removing radioactive ^{60}Co from nuclear effluents and Pb(II) and Cd(II) from drinking water. On the other hand, Cu(II)—chitosan complexes are used for the slow release of Cu(II) for soil conditioners and anti-fouling agents in paints [338].

(ii) Agriculture

Bacteria are important in determining the form and distribution of essential metals in soil and plants. They play an important role in modification, activation

and detoxification of heavy metals. Many factors of course, affect the form of metals, and thus their potential toxicity. These include, pH [339], chelating agents [340,341] and competition of other metal cations [342,343]. In plants, the roots which contain polyuronates on the surface of the cell are responsible for the cation uptake and transport of essential metals. It appears that these polyuronic components in plants can compete with humic acid and clay material in the soil.

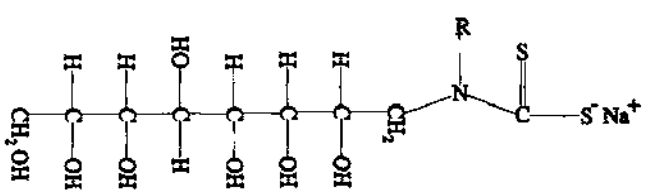
Certain organotin derivatives of carbohydrates have been introduced as agricultural chemicals due to their fungotoxicity [344].

(iii) Pharmaceutical

Carbohydrates are often highly soluble in water and are usually only weakly immunogenic and of low toxicity. For example, polysaccharide metal complexes, particularly of Mn(II) and Gd(III), have been used as magnetic resonance imaging carriers [345]. These properties are very useful in developing pharmaceutical agents. Thus, carbohydrate derivatives are good candidate compounds for the design of new drugs. One such possibility is the development of chelates for the removal of toxic metals or the uptake of essential metals. Based on our results with IdopA-containing oligosaccharides, it seems possible to synthesize compounds which will complex potentially toxic metals such as Ni(II), Cu(II) and Pb(II) and to promote elimination into urine via the kidneys. There are many considerations besides the overall thermodynamics, notably access to the in vivo metal pools and the kinetics of ligand exchange [346]. Experiments aimed at some of these questions are in progress in our group.

One example of this process is the use of the dithiocarbamates of an octose, notable *N*-4-methoxybenzyl-D-gluco-L-talooctamine dithiocarbamate (**XLIV**), for the removal of Cd(II) from Cd(II)-treated mice. The octose provides a large hydrophilic group which not only ensures water solubility but which, due to its size, prevents passage across the blood-brain barrier. The hydrophobic 4-methoxybenzyl group allows passage across cell membranes and helps to promote the mainly hepatic clearance of Cd(II) [347].

Clinically, the most important use of chelators is for the treatment of iron



R = 4-Methoxybenzyl Derivative of D-Gluco-L-Talooctamine Dithiocarbamate, (XLIV)

overload [348]. The potential market for a low cost, water soluble, effective iron chelator is enormous [349]. Since Fe(III) is predicted to form strong complexes with oxygen-based ligands (see Fig. 4) and since plants are known to use GalpA for iron uptake (see above), the background for development of carbohydrate-based iron chelators is clear.

E. CONCLUDING REMARKS

This review has shown that, with only a few notable exceptions, the chemistry of metal–sugar interactions has been little studied. This neglect is in part due to the complexity of carbohydrate chemistry. Recent developments in NMR technology have largely been responsible for the recent progress in this area. However, for these structural spectroscopic studies, it is advisable to use sugar glycosides as this eliminates the complications of mutarotations and redox chemistry. As was found for the chemistry of Fe(III) and Cu(II), this redox chemistry is probably crucial to the in vivo interactions of these metals with carbohydrates. Consequently, there is a large number of experimental studies which could be undertaken in this field. These range from uses in synthetic chemistry to industrial and pharmaceutical applications. Of particular interest is the search for oligosaccharides with at least four binding sites available for chelation. Such oligosaccharides should form much stronger complexes than most of the carbohydrates discussed in this review. With the notable exception of disaccharides (**XLIIIa**) and (**XLIIIb**), none of the carbohydrates exhibited four or more binding sites. At the same time, such chelating oligosaccharides should retain the desirable properties of high water solubility and low toxicity and immunogenicity, leading to many potential useful applications. Furthermore, the abundance of functional groups on carbohydrates allows for the manipulation of sites away from the binding sites so that other useful properties may be imparted to the molecules.

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