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Metal–saccharide chemistry and biology: saccharide complexes of zinc and their effect on metallothionein synthesis in mice

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Abstract

Monosaccharide (D-Fru, D-Gal, D-Glc, D-Xyl, and D-Rib) and disaccharide (Mal) complexes of Zn^{2+} were synthesised using different precursors and isolated in the solid state. These were found to be anionic with a Zn-to-saccharide ratio of 1:1 and 2:1 for monosaccharide and disaccharide complexes, respectively. Electrochemical behaviour in aqueous solution was studied by extensive cyclic voltammetric studies in the pH range 3.7–10.3. The effect of subcutaneously injected Zn–D-Fru, Zn–D-Gal and Zn–D-Glc complexes on the metallothionein synthesis in mice was found to be significant in the liver, but not in the brain.

Keywords: Complex, metal–saccharide; Zinc–saccharide complex; Metallothionein

1. Introduction

Saccharides form an interesting class of polymer molecules in biological systems. The presence of sugars in the skeleton of a variety of macromolecules including nucleic acids provides numerous potential metal-binding sites. The ability of metal ions to fold biopolymers has been long recognised; however, the extent to which this phenomenon occurs in biology has been appreciated only in recent years. The detailed three-dimen-

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sional structures of carbohydrates, and the role of metal ions in determining and regulating these structures are some of the greatest challenges open to chemists, particularly bioinorganic chemists [1]. Carbohydrate-binding proteins, lectins, have been shown to require metal ions such as calcium for binding [2]. Formation of Ni^{2+}–carbohydrate complexes has been demonstrated in human kidneys, indicating that such complexes may have biological relevance [3]. Thus metal–saccharide chemistry plays an important role in the cross-linking of various biomolecules. The interactions of alkali and alkaline–earth metal ions and other nontransition metal ions with saccharides and related compounds, resulting in the formation of saccharide adducts, have been investigated in great detail [4]. The interactions of transition metal ions with saccharides and their derivatives are limited to solution studies only [5]. The aminoglycosides of Co^{3+} and Ni^{2+}, and the D-lyxose complex of Mo^{6+}, are the only complexes that have been characterised crystallographically [6]. Our efforts during the past one-half decade resulted in the isolation, chemical characterisation, and studies of the biological relevance of several first-row transition metal–saccharide complexes [7]. Here we report the results of our studies on the synthesis and characterisation of Zn^{2+} complexes with simple saccharides, viz. D-fructose (D-Fru), D-galactose (D-Gal), D-glucose (D-Glc), D-xylose (D-Xyl), D-ribose (D-Rib), and maltose (Mal).

Metallothionein (MT) is a multi-regulated protein [8,9]. Heavy metals such as Cd, Zn, or Cu are primary inducers of the MT gene through the interaction of trans-acting factors sensitive to intracellular metal levels with the metal regulatory elements (MRE) of the promoter region [9]. The classical mammalian MT form binds a total of seven equivalents of divalent metal ions, and physiologically Zn is the most relevant metal. Therefore, we report here the potential biological activity of D-Fru, D-Gal, and D-Glc complexes of Zn^{2+} by means of their effect on liver and brain MT levels in mice.

2. Materials and methods

All solvents were distilled and dried before use by established procedures. $[NEt_4]_2[ZnCl_2Br_2]$ salt was prepared by the method of Gill and Taylor [10]. $ZnCl_2$ (Loba Chemie or Merck, India), D-Glc (Allied Chemicals), other saccharides (Aldrich Chemical Company), and sodium metal (Merck, India) were used as supplied without further purification. Adult male Swiss mice were used one week after their arrival at the laboratory. Mice were kept in groups of five per cage under standard conditions (lights on from 07.30 to 19.30 h, 22 °C, food, and water ad libitum). Laboratory mice food was from Panlab (Spain).

FTIR spectra of the free saccharides and the complexes were recorded in the 4000–400 cm^{-1} region in a KBr matrix on an Impact 400 Nicolet FTIR spectrophotometer. Dispersive IR spectra of the complexes and $ZnCl_2$ in CsI matrix were recorded in the region 4000–200 cm^{-1} on a Pye Unicam PU9512 infrared spectrophotometer. The electrochemical behaviour of these complexes was studied by means of cyclic voltammetry experiments of aqueous solutions, using the BAS 100B electrochemical analyser. A conventional three-electrode cell assembly of a hanging mercury drop electrode (HMDE) as the working electrode, platinum as the auxiliary electrode and

Ag/AgCl as the reference electrode, was employed. The solutions were made in doubly distilled water, and studies were carried out at varying pHs in the range pH 3.7–10.3 using HCl and NaOH to adjust the pH of the solution. Me_4NCl was used as the supporting electrolyte. Solutions were deoxygenated by purging argon for about 30 min, and the voltammograms of the argon-blanketed solutions were recorded in the range of -1.7 to -0.5 V, at a scan speed of 0.1 V/s. ^1H NMR spectra in D_2O solutions were recorded on a Varian XL300 spectrometer. C, H, and N analyses were done on Carlo Erba 1106 elemental analyser, and Zn and Na contents were determined by ICPAES on Plasmalab 8440.

3. Experimental

Synthesis of Zn^{2+}–saccharide complexes.—The complexes were synthesised using either $[NEt_4]_2[ZnCl_2Br_2]$ or $ZnCl_2$. The saccharides were used as sodium salts generated in situ in MeOH; disodium salts of monosaccharides (D-Fru, D-Gal, D-Glc, D-Xyl, and D-Rib), and tetrasodium salt of disaccharide (Mal) as reported earlier [7c].

A typical method for the preparation of Zn–D-Fru complex (**1**) *from $[NEt_4]_2[ZnCl_2Br_2]$.*—D-Fru (2.164 g, 12.0 mmol) was dissolved in 125 mL of anhyd MeOH. To this, freshly cut metallic sodium (0.557 g, 24.2 mmol) was added in pieces while stirring. About 30 min later, $[NEt_4]_2[ZnCl_2Br_2]$ (2.228 g, 4.0 mmol) in 15 mL of MeCN was added slowly with stirring to the methanolic solution of the sodium salt of D-Fru (1:3 metal-to-ligand ratio), which resulted in the formation of an off-white precipitate. The reaction mixture was stirred further for 1 day and then filtered by suction. The isolated precipitate was stirred in 30 mL portions of (*i*) anhyd MeOH and (*ii*) MeCN for 1 day each and finally (*iii*) 30 mL of hexane for about 18 h. However, this procedure was found to be ineffective in removing trace amounts of co-precipitated sodium- and/or ammonium-halide salts. Further purification by stirring in 30 mL of a 90:10 v/v MeOH–H_2O mixture for 1–2 h (thrice) and then in anhyd MeOH (twice) yielded the pure product of **1**.

An identical method of preparation was adopted for the synthesis of Zn–D-Gal complex (**2**). For the synthesis of the Zn–Mal complex (**6**), a 1:2 metal-to-δ-Mal ratio was employed using 2 mmol of $[NEt_4]_2[ZnCl_2Br_2]$.

Analytical data. Anal. Calcd for $C_{12}H_{24}Cl_3NaO_{13}Zn_2$ [Zn–D-Fru (**1**)]: C, 22.63; H, 3.77; Na, 3.62; Zn, 20.55. Found: C, 22.45; H, 3.69; Na, 3.71; Zn, 20.93. Anal. Calcd for $C_{12}H_{22}Cl_3NaO_{12}Zn_2$ [Zn–D-Gal (**2**)]: C, 23.29; H, 3.56; Na, 3.72; Zn, 21.15. Found: C, 22.99; H, 3.60; Na, 3.80; Zn, 20.88. Anal. Calcd for $C_{12}H_{22}O_{12}Cl_3NaZn_2 \cdot 4CH_3OH$ [Zn–Mal (**6**)]: C, 25.73; H, 5.09; Na, 3.08; Zn, 17.52. Found: C, 25.90; H, 4.51; Na, 3.39; Zn, 17.78.

A typical method for the preparation of Zn–D-Fru complex (**1a**) from $ZnCl_2$.—To the sodium salt of D-Fru in 120 mL of MeOH (2.16 g, 12.0 mmol of D-Fru; 0.57 g, 25 mmol of Na), $ZnCl_2$ (0.55 g, 4.0 mmol) in 20 mL of MeOH was added slowly with constant stirring, which resulted in the formation of the complex as an off-white precipitate. The stirring was continued for about 1 day under ambient conditions, after which the complex was isolated by filtration under suction. The isolated complex, which

is soluble in H_2O, was purified by stirring in 30 mL of a 90:10 v/v MeOH–H_2O mixture for 1–2 h (thrice) and finally with anhyd MeOH (twice). The complexes Zn–D-Gal (**2**), Zn–D-Glc (**3**), Zn–D-Xyl (**4**), and Zn–D-Rib (**5**) were synthesised and purified in an identical manner.

Analytical data. Anal. Calcd for $C_{12}H_{22}Cl_3O_{12}NaZn_2$: C, 23.29; H, 3.56; Na, 3.72; Zn, 21.15. Found: Zn–D-Fru (**1a**): C, 23.58; H, 3.59; Na, 3.63; Zn, 21.02; Zn–D-Gal (**2**): C, 23.26; H, 3.47; Na, 3.84; Zn, 21.29; Zn–D-Glc (**3**): C, 23.59; H, 3.67; Na, 3.90; Zn, 20.92. Anal. Calcd for $C_{10}H_{19}Cl_2O_{11}NaZn_2 \cdot 3CH_3OH$ [Zn–D-Xyl (**4**)]: C, 24.54; H, 4.88; Na, 3.62; Zn, 20.57. Found: C, 24.86; H, 4.60; Na, 3.67; Zn, 20.81. Anal. Calcd for $C_{10}H_{20}ClO_{12}NaZn_2 \cdot 3CH_3OH$ [Zn–D-Rib (**5**)]: C, 25.27; H, 5.18; Na, 3.73; Zn, 21.18. Found: C, 25.04; H, 4.75; Na, 3.74; Zn, 21.04.

Metallothionein (MT) synthesis in mice.—To study the effect of complexes Zn–D-Fru (**1a**), Zn–D-Gal (**2**), and Zn–D-Glc (**3**) on MT synthesis in mice, the animals were randomly assigned to the five experimental groups: (*i*) distilled water, (*ii*) $ZnCl_2$, (*iii*) Zn–D-Fru (**1a**), (*iv*) Zn–D-Gal (**2**), and (*v*) Zn–D-Glc (**3**). $ZnCl_2$ and the complexes were all dissolved in distilled water in order to inject into the animals 1 mL/100 g body weight subcutaneously at the Zn dose of 5 mg/kg. Complexes Zn–D-Gal (**2**) and Zn–D-Glc (**3**) were injected as mild suspensions after vortexing immediately before injection. The mice were killed by cervical dislocation 18 h after the administration of compounds, and the livers were rapidly removed and stored frozen at −80 °C until further dissection of the brains. For the MT assay, the livers and brains were thawed and homogenised in 5 vol of 10 mM Tris HCl, pH 8.2 with a Politron homogeniser at a low speed. Homogenates were centrifuged at 16,000*g* for 40 min at 4 °C, and the supernatants stored at −20 °C. MT was measured by ELISA [11a] using polyclonal antibodies that specifically cross-react with the MT-I and MT-II isoforms [11]. Samples were assayed in the same assay to avoid interassay variations. Zn was measured by atomic-absorption spectroscopy (AAS). Results were evaluated with one-way ANOVA, followed by the SNK multiple-range procedure.

4. Results and discussion

The synthesis of complexes using sodium salts of saccharides enhances their reactivity towards metal-ion coordination through deprotonated hydroxyl groups in nonaqueous solvents and yield water-soluble complexes. Metal–saccharide interactions are known in the literature, both in solution and solid states, as saccharide adducts [4,5]; however, our group has recently reported complexes where saccharides bind metal ions through the deprotonated hydroxyl groups [7]. Literature reports include the adducts of zinc group metal ions with a composition of $M(Sacch)X_2 \cdot nH_2O$ (where Sacch = saccharide, X = Cl or Br) for Zn^{2+}, Cd^{2+}, and Hg^{2+} [4l–n]. The dimeric complexes, reported here, prepared from different Zn^{2+} precursors, possess saccharides coordinated to Zn^{2+} through deprotonated hydroxyl groups.

FTIR studies.—The FTIR spectra of Zn^{2+}–saccharide complexes were compared with those of the corresponding free saccharides in the region 4000–400 cm^{-1} and with $ZnCl_2$ in the far-IR region 400–200 cm^{-1}. The spectra of the sodium salts of

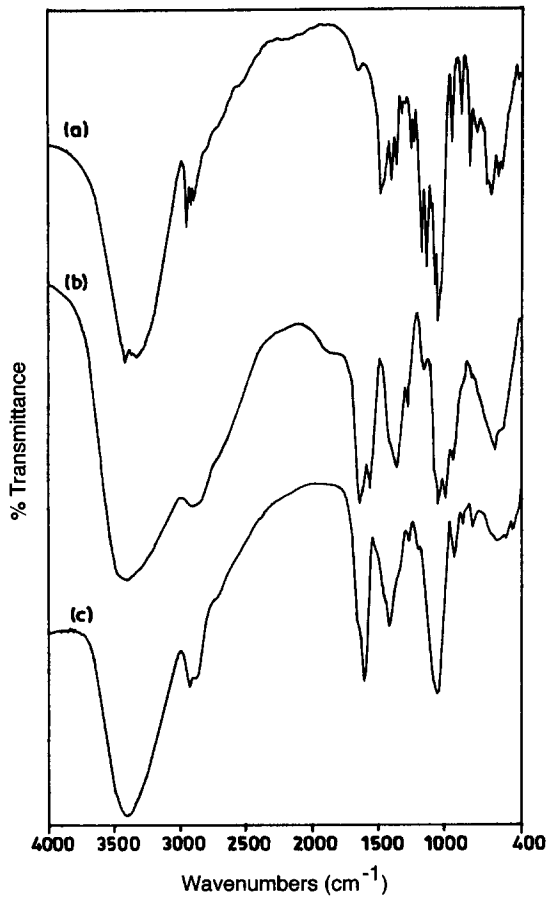

Fig. 1. FTIR spectra of (a) D-Glc, (b) D-Glc sodium salt, and (c) Zn–D-Glc complex (**3**) in KBr matrix.

saccharides, as well as all of the complexes, showed extensive rearrangement of their hydrogen-bonding network due to ionisation of the saccharides, and these were characterised by the presence of broad and merged bands in all the cases. Fig. 1 shows a comparison among free D-Glc, its sodium salt, and the Zn–D-Glc complex (**3**). The spectra of all the complexes showed broad bands in the O–H and C–H stretching regions, indicating a merging of individual bands and thereby making the assignments of the individual vibrational modes difficult. The stretching vibrations of the intermolecular hydrogen-bonded O–H groups of the free saccharides in the region 3500–3200 cm^{-1} were affected upon ionisation and exhibited a broad but nearly symmetrical band at $\sim$ 3400 cm^{-1} with a shoulder at $\sim$ 3250 cm^{-1}. The strongly coupled ring vibrational frequencies for bending modes COH, CH_2 and CCH of the free saccharides (1460–1340 cm^{-1}) showed merging at 1400 cm^{-1} upon complex formation; however, this region showed several medium-intensity absorption bands in case of alkaline–earth and zinc group metal–saccharide adducts [4g–m]. Similarly, the C–O and C–C stretching

vibrations in the region 1140–990 cm^{-1} were also merged at ~ 1050 cm^{-1} upon complex formation, in contrast to the sharp bands observed for the free saccharides and other metal–saccharide adducts. The anomeric region (950–500 cm^{-1}) showed very weak marker bands of mostly α anomer, even in those cases where the β form of saccharide was used for synthesis (e.g., β-D-Fru). The presence of the α anomer in the complexes could be attributed to its thermodynamic stability as compared to the β anomer, which is a kinetically controlled product [12]. It is well known that Na^+ has no effect on the anomeric equilibria and also that the proportion of the α form increases in solution in the presence of complexing cations [4d]. Therefore, the conversion of β to α form was facilitated by the complexation of Zn^{2+} and is suggestive of the involvement of the 1-OH in complexation. This type of saccharide binding to Zn^{2+} via 1-OH and 2-OH groups is reported for the alkaline–earth metal–D-Glc adducts [4h]. In the literature, the saccharide adducts of Zn^{2+}, Cd^{2+}, Mg^{2+}, and Ca^{2+} exhibited prominently the presence of the α anomer, while that of Hg^{2+} was shown to possess a mixture of α and β anomers [4h–m]. Thus it can be observed that the complexation in the present case influenced almost all the modes of vibration resulting in *merging and broadening* of bands in contrast to adducts which resulted in *splitting and shifting* of these. The merging and broadening of bands was found to be a common feature of transition metal–saccharide complexes synthesised similarly using sodium salts of the saccharides [7].

In the far-IR region (400–200 cm^{-1}) the complexes exhibited a medium-intensity broad band at 277 cm^{-1}, corresponding to ν_{Zn-Cl} as compared to that of $ZnCl_2$, which exhibited a very strong band at 291 cm^{-1}, thus suggesting the involvement of Cl^- binding in the complexes.

Cyclic voltammetric studies.—A similar voltammetric behaviour was observed for all the complexes studied [Zn–D-Fru **(1a)**, Zn–D-Gal **(2)**, and Zn–D-Glc **(3)**] and was found to be reproducible in the acidic-to-basic pH range, or vice versa, irrespective of the starting pH. These were compared under identical conditions with those of $ZnCl_2$ alone

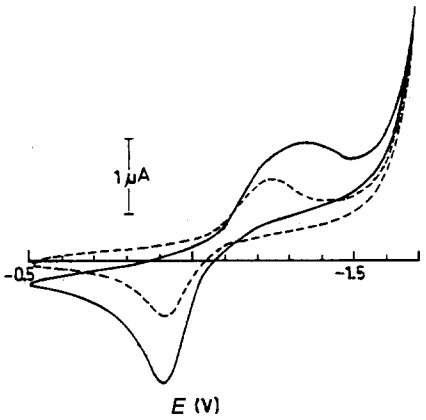

Fig. 2. Cyclic voltammograms of (solid line) the Zn–D-Gal complex **(2)** and (dashed line) the Zn–D-Gal complex **(2)** with five equivalents of added D-Gal, both at pH 8.7.

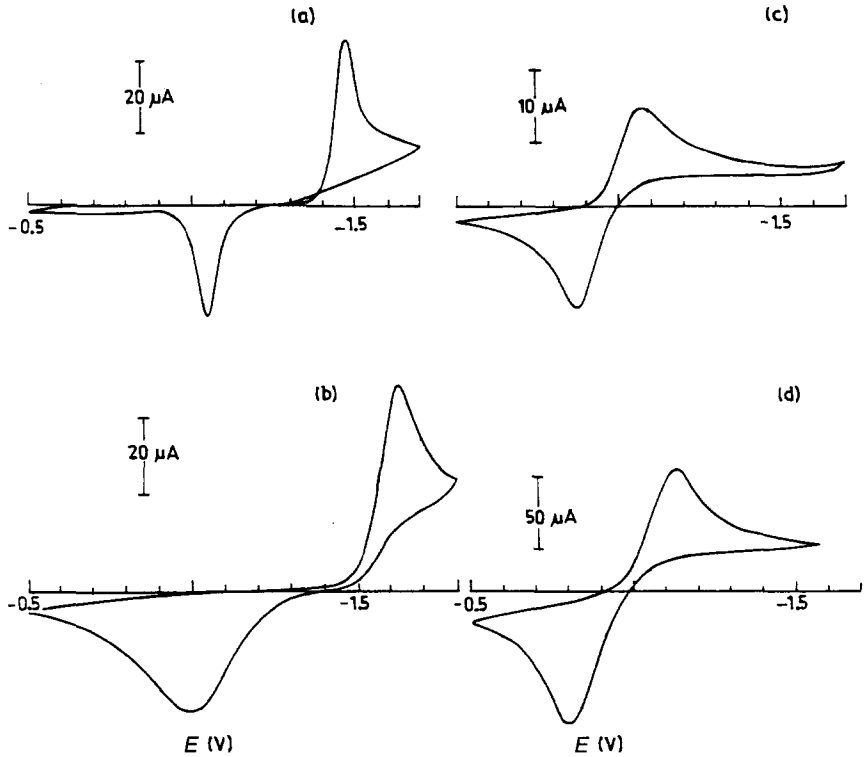

Fig. 3. Cyclic voltammograms of the Zn–D-Gal complex (**2**) at pH values (a) 9.9, (b) 10.3, (c) 7.3 and (d) 5.8.

and also with those after the addition of five equivalents of free saccharide to complexes and $ZnCl_2$. The following discussion is presented in the case of complex Zn–D-Gal (**2**), as all the complexes exhibited a similar behaviour.

Freshly prepared aqueous solutions of these Zn^{2+}–saccharide complexes exhibited a pH > 8.5 compared to a pH < 7 for $ZnCl_2$, indicating the involvement of deprotonated hydroxyl groups of saccharide in the complexation. The cyclic voltammogram of Zn–D-Gal (**2**) at pH 8.7 (Fig. 2) showed an anodic peak (E_p^a) at -0.91 V and a cathodic peak (E_p^c) at -1.36 V. It is known that in the presence of sugars, electrochemical deposition of Zn from Zn^{2+} solutions is favoured through the formation of adsorbed Zn species [12]. This was confirmed from the observation that, on the addition of five equivalents of free D-Gal to the Zn–D-Gal complex (**2**), at pH 8.7 (Fig. 2), the E_p^c was shifted towards a positive potential (-1.23 V), whereas the E_p^a was almost unaffected (-0.92 V). Since the ligand-exchange reactions are rapid in the case of Zn^{2+}, some $Zn(OH)_2$ precipitate was formed due to the presence of hydroxide ions in solution. On increasing the pH to 9.9 (Fig. 3a), the E_p^c and E_p^a shifted to further negative potentials. The E_p^c (-1.48 V) was comparable to that of $ZnCl_2$ at similar pH (-1.47 V). However, the cathodic shift of E_p^a (-1.04 V), as compared to that of $ZnCl_2$ (-0.97 V) indicated the ease in oxidation process from adsorbed species in the case of the Zn–D-Gal complex (**2**).

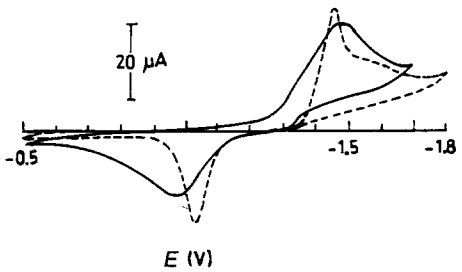

Fig. 4. Cyclic voltammograms of (solid line) $ZnCl_2$ and (dashed line) $ZnCl_2$ with five equivalents of added D-Gal, both at pH 9.9.

The addition of five equivalents of D-Gal to the Zn–D-Gal complex (**2**) (at pH 9.9) produced further cathodic shifts of E_p^c (−1.51 V) and E_p^a (−1.07 V). These changes were indicative of the involvement of deprotonated hydroxyls of saccharide units and/or free OH^- ions, and thereby resulted in an easy oxidation of $Zn \rightarrow Zn^{2+}$. The cathodic and anodic peaks were characteristic of adsorption processes in the case of the Zn–D-Gal complex (**2**) (Fig. 3a) but not in the case of $ZnCl_2$ alone at identical pH (Fig. 4). However, the addition of five equivalents of free D-Gal to $ZnCl_2$ exhibited such adsorption characteristics (Fig. 4) indicating some interaction of D-Gal with Zn^{2+} through deprotonated hydroxyl groups. The shifts observed in E_p^c and E_p^a upon the addition of D-Gal to $ZnCl_2$ were indicative of a possible complex formation and further confirmed that the redox processes were favoured upon adsorption.

On increasing the pH to 10.3, the E_p^c of the Zn–D-Gal complex (**2**) shifted to −1.62 V and E_p^a to −0.99 V (Fig. 3b). Addition of five equivalents of free D-Gal to Zn–D-Gal (**2**) shifted E_p^c and E_p^a to −1.66 and −0.94 V, respectively. The shift of E_p^c is consistent with an increase in the overall negative charge on the species. The shift of E_p^a is suggestive of the oxidation of $Zn \rightarrow Zn^{2+}$ becoming difficult in the absence of adsorption processes as seen from the nature of the redox waves at this pH. The results are in agreement with the fact that a maximum number of four saccharide ligands can be coordinated to each Zn^{2+} ion in alkaline solution [13].

On decreasing the pH of a freshly prepared solution of the Zn–D-Gal complex (**2**) from pH 8.7 to near-physiological pH 7.3 (Fig. 3c), the E_p^a and E_p^c were shifted towards positive potentials ($E_p^a = -0.87$ V, $E_p^c = -1.07$ V). Under similar conditions $ZnCl_2$ exhibited an E_p^a at −0.80 V and an E_p^c at −1.13 V. The positions of E_p^a and E_p^c of the Zn–D-Gal complex (**2**), as compared to that of $ZnCl_2$, indicate a weak interaction of neutral D-Gal with Zn^{2+} at physiological pH. This is attributed to the higher pK_a values of hydroxyl groups of the saccharides. A weak interaction of D-Gal with Zn^{2+} was also observed upon the addition of five equivalents of free D-Gal to $ZnCl_2$ at similar pH, which showed an identical behaviour to that of the Zn–D-Gal complex (**2**). Addition of five equivalents of D-Gal to the Zn–D-Gal complex (**2**) did not show appreciable changes in the voltammetric behaviour.

Further lowering the pH to 4.6 through 5.8 (Fig. 3d) shifted the E_p^c of the complex to cathodic potentials (−1.28 V), and the E_p^a to anodic potentials (−0.75 V). These changes were indicative of an almost complete protonation of saccharide units and

replacement by Cl^- in which $Zn^{2+} \rightarrow Zn$ reduction takes place at more cathodic potentials. Similar behaviour was observed with $ZnCl_2$ under identical conditions. Moreover, the addition of even five equivalents of free saccharide to a solution of $ZnCl_2$ at pH ≤ 4, did not indicate any changes in the E_p^a or E_p^c, confirming the absence of any interaction between Zn and added saccharide units in acidic conditions. However, such interactions are possible in case of carboxylate-containing saccharides owing to their lower pK_a values [14].

In summary, the cyclic voltammetric experiments demonstrate the presence of complexes in freshly prepared aqueous solutions. The interactions of saccharide with Zn^{2+} are strengthened under alkaline conditions owing to deprotonation of several hydroxyl groups and formation of mixed saccharide and the hydroxy-bound anionic Zn^{2+} complex, $[Zn(Gal)(OH)_m]^{n-}$. Under acidic conditions (pH < 5), these interactions are absent. However, a weak interaction persists at physiological pH in conformity with the lower stability of Zn^{2+}–saccharide complexes [14].

^1H NMR studies.—The ^1H NMR spectra of the complexes recorded in D_2O exhibited peaks in a broad envelope in the region 3.5–4.5 ppm in contrast to sharp peaks for free saccharides, and hence they could not be assigned to individual protons. However, the overall position of the peaks was marginally shifted downfield upon complexation. Peaks for the presence of both α and β anomers suggest a spontaneous mutarotation in solution, resulting in an equilibrated mixture of anomers.

Nature of the products.—In order to synthesise Zn^{2+}–saccharide complexes of diverse nature, either $[NEt_4]_2[ZnCl_2Br_2]$ in MeCN or $ZnCl_2$ in MeOH was used as the starting material. Initially complex formation from $[NEt_4]_2[ZnCl_2Br_2]$ involves the removal of Br^- in nonaqueous media to give complexes of the type Na[Zn(Sacch)(OMe)Cl], along with trace amounts of co-precipitated sodium- and/or tetraethylammonium-halide salts, as reported earlier [7h].

$$\underset{\text{(MeCN)}}{[NEt_4]_2[ZnCl_2Br_2]} + 3Na_2Sacch \xrightarrow{\text{Stir (RT)}} \underset{\text{(MeOH, in situ)}}{Na[Zn(Sacch)(OMe)Cl]} + m\underset{(X = Cl/Br)}{NaX} + n\underset{(X = Cl/Br)}{NEt_4X}$$

$$2\,Na[Zn(Sacch)(OMe)Cl] + m\underset{(X = Cl/Br)}{NaX} + n\underset{(X = Cl/Br)}{NEt_4X} \xrightarrow[(MeOH–H_2O)\,(90:10)]{\text{Stir (RT)}} Na[Zn_2(Sacch)_2Cl_3]$$

However, these complexes are converted into dimeric form, as shown above, by stirring the reaction products in a 90:10 v/v MeOH–H_2O mixture, due to the destruction of methoxide in water, and the addition of another molecule. The complexes formed from $ZnCl_2$ and purified directly from a 90:10 v/v MeOH–H_2O mixture yield similar products. Based on the elemental analysis, the compounds were assigned the molecular formula, $Na[Zn_2(Sacch)_2Cl_3] \cdot nH_2O$ [where Sacch = D-Fru (**1**) or D-Gal (**2**), or D-Glc (**3**); $n = 1$ for Zn–D-Fru (**1**) and $n = 0$ for Zn–D-Fru (**1a**), Zn–D-Gal (**2**), and Zn–D-Glc (**3**)]. The complexes Zn–D-Xyl (**4**), Zn–D-Rib (**5**), and Zn–Mal (**6**) were assigned the molecular formulae $Na[Zn_2(Xyl)_2(OH)Cl_2] \cdot 3CH_3OH$, $Na[Zn_2(Rib)_2(OH)_2Cl] \cdot 3CH_3OH$ and $Na[Zn_2(Mal)Cl_3] \cdot H_2O \cdot 4CH_3OH$, respectively. Thus it can be seen that

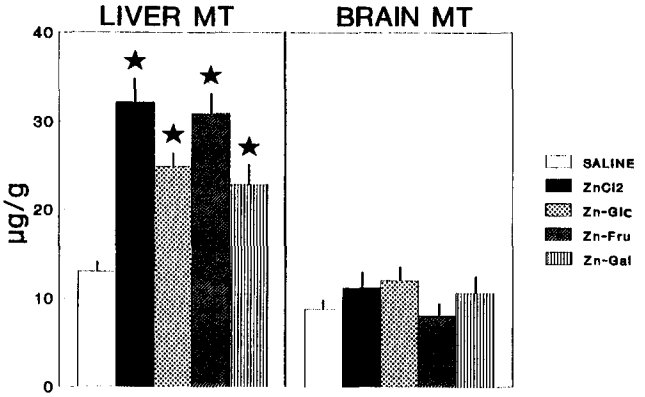

Fig. 5. Effect of Zn^{2+}–saccharide complexes on liver and brain metallothionein (MT) levels as compared to $ZnCl_2$. Results are mean ± SE. $n = 9$ and $n = 6$ for the liver and brain, respectively. ★$p < 0.05$ vs. saline-injected mice.

both of the procedures predominantly yield identical tetrahedral Zn^{2+}–saccharide complexes with a 1:1 Zn-to-saccharide ratio for monosaccharide complexes (**1–5**) and a 2:1 ratio for the disaccharide complex Zn–Mal (**6**). The thermodynamically more stable α anomer was found to be preferred for binding in the complexes isolated in the solid state, whereas a spontaneous mutarotation in aqueous solution results in the presence of an equilibrated mixture of both the anomers. As proposed in the formulae, the binding of Cl^- in the complexes was confirmed by the presence of ν_{Zn-Cl} in the far-IR spectra.

Effect of Zn^{2+}–saccharide complexes on metallothionein (MT) synthesis in mice.— Liver and brain MT levels are shown in Fig. 5. The effect of the Zn compounds on liver MT levels was highly significant, as revealed by one-way ANOVA ($p < 0.0001$). Post-hoc comparisons of the means with the SNK procedure indicated that $ZnCl_2$ and the three Zn^{2+}–saccharide complexes [Zn–D-Fru (**1a**), Zn–D-Gal (**2**), and Zn–D-Glc (**3**)] increased significantly the liver MT levels (p at least < 0.05), and that the induction was comparable in the four groups (those injected with Zn compounds) since no statistical significances were obtained when compared between them. In the case of brain MT levels, although some trends could be observed, no statistical significances were achieved. With regard to the liver and brain cytosolic Zn levels, Zn treatments tended to increase marginally the liver Zn levels, but no statistical significances were achieved (data not shown).

The complexes Zn–D-Fru (**1a**), Zn–D-Gal (**2**), and Zn–D-Glc (**3**) were capable of inducing liver MT synthesis, indicating that these complexes possess biological significance and perhaps could be considered clinically as substitutes for Zn salts. However, the studies in this direction deserve further attention. Gross examination of the animals did not indicate any sign of toxicity. The body weight and liver weights were not affected by administering these complexes. It is well known that Zn induces MT synthesis through trans-acting factors interacting with specific metal regulatory elements (MRE) of the promoter region [9a,b]. Thus, for the Zn^{2+}–saccharide complexes to induce liver MT synthesis, the Zn moiety should dissociate from the complex either

extra- or intra-cellularly and then interact with the trans-acting factor(s) sensitive to cellular Zn levels. Zn^{2+}–saccharide complexes have low hydrolytic stability [14], and cyclic voltammetric studies demonstrated a weak interaction of saccharide with Zn^{2+} at physiological pH. It is likely that such weakly bound saccharide is replaced by albumin in the blood, and the corresponding albumin-bound Zn^{2+} behaves normally regarding the liver Zn absorption [15] and consequent MT induction. The albumin-bound Zn^{2+} is not expected to reach the brain cells, and hence brain MT levels were not affected by these complexes, which is consistent with the effect of $ZnCl_2$ alone, i.e., no MT induction. It is well known that Zn administered peripherically does not enter into the brain significantly because of the blood–brain barrier, and consequently does not induce brain MT synthesis [16].

5. Conclusions

Our earlier efforts in the field of metal–saccharide chemistry has clearly demonstrated the ability of polyhydroxy compounds such as saccharides in binding transition metal ions [7]. This paper demonstrates the complex formation between Zn^{2+} and deprotonated hydroxyls of simple saccharides in the solid state, whereas in aqueous solution the complexation is favoured at pH > 8 as demonstrated by cyclic voltammetric experiments. Since it is known that Ni^{2+}–saccharide complexes occur in biological systems, it is believed that similar Zn^{2+}–saccharide complexes may also have an existence in biological systems. The Zn^{2+} is presumably released from the saccharide complexes due to direct competition with albumin at physiological pH and has an expected effect on the MT synthesis in the liver and brain. Zinc is an essential element in biological systems, and its presence at active sites of many hydrolytic enzymes optimises its ability to lower the pK_a of coordinated oxygen-containing ligands. Therefore, the nontoxic, water-soluble, anionic, dimeric Zn^{2+}–saccharide complexes reported in this paper appear to have biological significance and could serve as potential dietary supplements for zinc deficiency.

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