Transition metal saccharide chemistry and biology: syntheses, characterization, solution stability and putative bio-relevant studies of iron–saccharide complexes

Chebrolu P. Rao [a,*], K. Geetha [a], M.S.S. Raghavan [a], A. Sreedhara [a], K. Tokunaga [b], T. Yamaguchi [b], V. Jadhav [c], K.N. Ganesh [c], Thanuja Krishnamoorthy [d], Kolluru V.A. Ramaiah [d], R.K. Bhattacharyya [e]

[a] *Bioinorganic Laboratory, Department of Chemistry, Indian Institute of Technology, Bombay, Powai, Mumbai 400 076, India*
[b] *Department of Chemistry, Fukuoka University, Fukuoka, Japan*
[c] *Synthetic Organic Division, National Chemical Laboratories, Pune 411 008, India*
[d] *Department of Biochemistry, School of Life Sciences, University of Hyderabad, Hyderabad 500 046, India*
[e] *Radiation and Biochemistry Division, Bhabha Atomic Research Centre, Mumbai-400 085, India*

This paper is dedicated to Professor Stephen J. Lippard.

Abstract

A number of Fe(III) complexes of saccharides and their derivatives, and those of ascorbic acid were synthesized, and characterized by a variety of analytical, spectral (FT-IR, UV–Vis, EPR, Mössbauer and EXAFS), magnetic and electrochemical techniques. Results obtained from various methods have shown good correlations. Data obtained from EPR, magnetic susceptibility and EXAFS techniques could be fitted well with the mono-, di- and trinuclear nature of the complexes. The solution stability of these complexes has been established using UV–Vis absorption and cyclic voltammetric techniques as a function of pH of the solution. Mixed valent, Fe(II,III) ascorbate complexes have also been synthesized and characterized. Reductive release of Fe(II) from the complexes using sodium dithionite has been addressed. In vitro absorption of Fe(III)–glucose complex has been studied using everted sacs of rat intestines and the results have been compared with that of simple ferric chloride. Fe(III)–saccharide complexes have shown regular protein synthesis even in hemin-deficient rabbit reticulocyte lysate indicating that these complexes play a role that is equivalent to that played by hemin in order to restore the normal synthesis of protein. These complexes have exhibited enhanced DNA cleavage properties in the presence of hydrogen peroxide with pUC-18 DNA plasmid.

Keywords: Transition metal saccharide; Solution stability; Putative bio-relevant studies; Iron–saccharide complexes; Electrochemical studies; Magnetic studies; EXAFS spectra

1. Introduction

The quest for identification of soluble and bio-available complexes of iron suitable for nutritional supplementation to humans is a long-standing problem and continues to provide enough impetus and input for basic research in this direction. We have been attempting to look at this aspect in terms of transition metal saccharide chemistry and biology due to the importance of the interaction of transition metal ions with saccharides and their derivatives, in chemistry as well as in biology. Furthermore, the saccharides are qualified as potential ligands not only due to their extensive presence in the biological systems, but also due to the presence of multi-hydroxy functionality and well-defined stereo-chemistry suitable for metal coordination. Added to this, the saccharides are the most

abundant class of compounds by weight in the biosphere and are highly water-soluble with weak immunogenicity and low toxicity. Ever since the pioneering work of Saltman in the 1960s regarding the iron–saccharide complexes [1], and the chelating ability studies of sugars by Davis and Deller [2], there has been a large gap in the literature relating the knowledge of these complexes. This is partly attributable to the high pK_a and low coordinating abilities of the –OH groups of saccharides. Interaction of iron with polygalacturonic acid and citric acid has become an important aspect in explaining the mechanism of uptake and transport of iron [3]. Later, several groups, including those of Burger and Nagy, have reported the oligo-to-polynuclear complex formation of iron with simple as well as derived saccharides [4]. Recently, Hegetschweiler's group has reported iron complexes of hydroxy compounds including those of inositols [5]. The studies indicated that these ligands interact through deprotonated hydroxyl groups in forming complexes with iron rather than just forming the adducts, as reported earlier in the literature regarding alkali and alkaline earth metal ions [6]. However, a systematic development of transition metal saccharide chemistry has not taken place in the literature until recently. During the past several years our group has contributed immensely to the growth of the subject of transition metal–saccaharide chemistry and biology [7]. This includes the development of simple synthetic strategies in the case of VO^{2+}, Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and MoO_2^{2+}. The studies comprised the isolation, characterization and solution stability of the complexes, including those of iron. Furthermore the biologically relevant aspects of the saccharide complexes of Cr(III) [8], VO^{2+} [9] and Zn(II) [10] have also been studied. As a continuation of our on-going efforts, we discuss here the synthesis, characterization, solution stability and bio-related properties of the complexes of some iron saccharides and their derivatives. The paper also emphasizes various data correlations among the iron-saccharide complexes reported by us.

2. Experimental

Materials used and methods adapted are the same as in our earlier paper in this field [11c]. Therefore, only those methods which were not dealt with earlier are discussed in this section.

2.1. Synthesis

All the complexes were synthesized using ferric chloride and in situ generated disodium salt of saccharides and their derivatives as reported earlier [11].

Fe(III)–D-ribose complex: D-ribose (1.35 g, 9 mmol) was dissolved in 60 ml of methanol to which 0.414 g (18 mmol) of sodium was added in small pieces. To this, 40 ml of methanolic solution of ferric chloride 0.486 g (3 mmol) was added to give an immediate yellow precipitate. After stirring for 1 h, a brown-colored solid was obtained. The reaction mixture was stirred for 1 day and the product was separated. The isolated product

Table 1
Table comprising the analytical data and the proposed molecular formulae for iron–saccharide complexes **1**–**7**

Compound no.	Formula	Analysis (%) [a]			
		Fe	Na	C	H
1	$Na_2Fe_2C_{15}H_{26}O_{17}\cdot 4H_2O$	c 16.12	6.59	24.90	5.23
	$\{Na_2[Fe_2(D\text{-rib})_2(OH)_2(H_2O)_2]\}$	o 15.70	6.40	25.40	4.84
2	$Na_2Fe_3C_{15}H_{29}O_{17}\cdot 2H_2O$	c 23.32	5.54	24.65	4.00
	$\{Na_2[Fe_3(D\text{-xyl})(OH)_2(H_2O)_2]\}$	o 22.90	6.29	24.63	4.10
3	$Na_2FeC_{12}H_{21}O_{15}$	c 10.91	8.90	27.74	4.13
	$\{Na_2[Fe_2(galactourionate)_2(OH)]\}$	o 11.01	9.00	28.40	4.17
4	$Na_3Fe_2C_{13}H_{16}O_{16}\cdot H_2O$	c 18.00	11.02	25.33	2.45
	$\{Na_3[Fe_2(citrate)_4(MeOH)(H_2O)]$	o 17.90	11.06	25.00	2.42
5	$Na_4Fe_2C_{21}H_{40}O_{16}N_3\cdot 6H_2O$	c 12.10	9.80	28.40	5.68
	$\{Na_4[Fe_2(N\text{-meglucosamine})_2(OH)]\}$	o 12.38	10.19	27.90	5.86
6	$Na_3Fe_2C_{24}H_{26}O_{25}\cdot 2H_2O$	c 12.05	7.41	30.60	3.09
	$\{Na_3[Fe_2(asc)_4(\mu\text{-}OH)(H_2O)_2]\}$	o 12.00	7.41	30.94	3.25
7	$Na_3Fe_3C_{36}H_{41}O_{38}\cdot 3H_2O$	c 11.96	4.98	31.26	3.60
	$\{Na_3[Fe_3(asc)_6(OH)_2(H_2O)_3]$	o 12.21	5.03	31.51	3.45

[a] c: calculated; o: observed.

was purified by dissolving in water and re-precipitating the same with ethanol. This purification process was repeated three times successively. The product **1** thus obtained was washed with ether and dried under vacuum. A similar procedure was adopted in synthesizing Fe(III)–D-xylose (**2**), Fe(III)–D-galacturionate (**3**), Fe(III)–citrate (**4**), Fe(III)–*N*-Me-glucosamine (**5**), Fe(III)–ascorbate (**6**), Fe(II,III)–ascorbate (**7**) complexes with product yields in the range 42 to 58%, derived based on the iron content. The results of elemental analysis of these complexes are given in Table 1. In this paper we have also utilized the data corresponding to the other iron–saccharide complexes that we reported earlier [11c], viz., Fe(III)–D-glucose (**8**), Fe(III)–L-sorbose (**9**), Fe(III)–D-fructose (**10**), Fe(III)–D-mannose (**11**), Fe(III)–D-galactose (**12**), Fe(III)–maltose (**13**), Fe(III)–lactose (**14**), Fe(III)–sucrose (**15**).

2.2. EXAFS and XANES

These spectra were measured around the Fe K-edge (7.11 keV) in the transmission mode using the BL10B station at the Photon Factory in the National Laboratory for High Energy Physics. Broad band synchrotron radiation was monochromatized by an S(311) channel-cut crystal and was passed through a sample placed between the first and second ionization chambers, which were filled with N_2 and N_2 (85%) + Ar(15%) gases, respectively. Experimental details were similar to those reported in the literature [12].

2.3. Reductive release of iron

To a solution of Fe(III)–saccharide complex (1 mmol) in water, reducing agent (sodium dithionite or paramethylaminophenol or cysteine) was added (1–100 mmol). To this solution was added 2 ml of 1,10-phenanthroline (200 mg in 20 ml of *N*,*N*-DMF) which was then stirred for 1 min and the solution was treated with an aqueous suspension of excess EDTA (100 mg) in order to prevent further reduction. The mixture was diluted to 50 ml and the pH was adjusted to 4.0 with acetic acid. The volume of the solution was made up to 100 ml. After filtering the solid EDTA, the Fe(II) content was determined spectrophotometrically using the absorbance exhibited at 510 nm. Cyclic voltammograms of the complexes at different time intervals were recorded after addition of 3 equiv. of sodium dithionite in the presence of tetraethylammonium chloride as supporting electrolyte. All the electrochemical measurements were carried out using Pt as working electrode.

2.4. In vitro absorption

^{59}Fe(III)–D-glucose and ^{59}Fe(III)–D-fructose complexes were prepared using $^{59}FeCl_3$. In vitro absorption of ^{59}Fe–saccharide complexes across intestinal tissue was studied in everted jejunal segments of the small intestines of rat. The everted segments of 5 cm length were filled with 0.2 ml of Krebs Ringer's (phosphate buffer, pH 7.0), tied at both ends and incubated at 37°C in 2.0 ml Krebs Ringer's (phosphate buffer, pH 7.0) containing the labeled complex. After 30 min of incubation the sacs were removed and radioactivity was counted on a Beckman LS100 scintillation counter. ATP (0–100 mM) was added to the system in other experiments in order to evaluate the influence of ATP on the transport process.

2.5. Protein synthesis

Hemin-deficient rabbit reculocyte lysates were prepared and the protein synthesis assays were carried out at 30°C for 60 min by the incorporation of labeled leucine into TCA-precipitable protein at specified time points in 5 μl aliquots as previously mentioned in the case of vanadium complexes. Experiments were carried out both in the presence and in the absence of hemin. All experimental conditions adapted are the same as those reported earlier [9e], except that the iron–saccharide complexes were used in place of VO^{2+}–saccharide complexes in all the studies.

2.6. DNA cleavage studies

The in vitro cleavage of pUC-18 DNA structure was studied by agarose gel electrophoresis in the absence and in the presence of H_2O_2 and mercapto ethanol (ME). A typical composition of a 20 μl reaction mixture includes tris–HCl buffer at pH 7 (20 mM), pUC-18 DNA (0.2 μg), H_2O_2 (4 mM), ME (0.5 mM) and the iron compound 2 μl (from 25 mg of compound/ml solution which corresponds to a concentration range of 15–50 mM and finally, giving rise to a compound concentration of 5 to

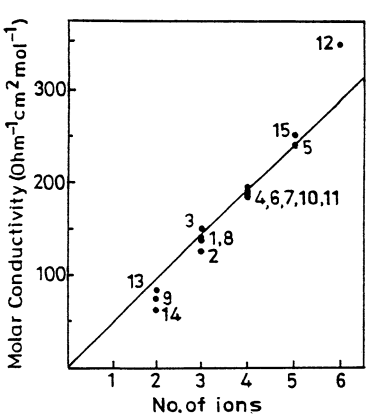

Fig. 1. Plot of molar conductivity vs. total number of ions in iron–saccharide complexes. The numbers given in the plot indicate the compound numbers.

15 nM in the DNA reaction mixture). Appropriate blank experiments were performed using DNA, H_2O_2 and ME. Other experimental procedures were the same as those given in our earlier papers reported for Cr(III)– and VO^{2+}–saccharide complexes, respectively [8,9a].

3. Results and discussion

Complexes **1**–**7** are highly soluble in water and insoluble in many organic solvents. These complexes exhibited aqueous molar conductivity that correlates well with the iron–saccharide complexes as shown in Fig. 1. Thus **1**–**3**, and **4**, **6**, **7**, and **5** have exhibited 1:2, 1:3 and 1:4 electrolyte behavior, respectively. Furthermore, the proposed compound formulae (Table 1) agree well with the conductivity behavior of the complexes. All the complexes have been characterized extensively both in the solid and in the solution states, and have been studied for their stability in solution. Putative bio-relevances of these complexes have been explored and the corresponding results are reported in this paper.

3.1. Characterization

Complexes **1**–**7** have been characterized by elemental analysis, FT-IR, UV–Vis, EPR, and Mössbauer spectra, in addition to magnetic susceptibility measurement (in the solid state) and electrochemical behavior (in the solution state). Complexes **8**–**12** were studied by EXAFS and XANES spectra. All these studies have led to the confirmation of the compounds reported in Table 1.

3.2. FT-IR spectra

FT-IR spectra of complexes **1**–**7** are broad and are reminiscent of other iron–saccharide complexes [11b,c]. The bands appearing at 1608 and 1405 cm^{-1} in **3** are assignable to the ν_{asym} and ν_{sym} vibrations, respectively of the carboxylate function, indicating that this group binds in mono-dentate fashion. The spectrum of **5** exhibited a band at 1530 cm^{-1} indicating that the amino group is not involved in the complexation. Bands observed at 780 and 806 cm^{-1}, respectively, in **6** and **7** are assignable to the ν_{asym} vibration of the Fe–O–Fe unit present in these complexes based on literature comparisons [12]. However, a definite assignment of such a vibration is only possible through isotope labeling studies. All the other features observed in the spectra were similar to those reported earlier for other iron–saccharide complexes [11].

Table 3
Solid state reflectance data for iron–saccharide complexes

Compd	λ (nm)
1	300, 340, 460, 450
2	300, 375, 450, 550
3	300, 375, 460, 575
4	325, 360, 450, 580
5	310, 380, 460, 625

3.3. UV–Vis spectra

Solution absorption spectra of **1**–**7** were measured in water, methanol and *N*,*N*-DMF, and the corresponding data regarding the band positions and molar absorptivities are listed in Table 2. The data are indicative of the involvement of solvent molecules in the coordination sphere of iron–saccharide complexes. While the spectra of **1**–**5** were characteristic of high-spin Fe(III), those of **6** and **7** were characteristic of the presence of both the Fe(II) and Fe(III) centers in the complexes. The presence of Fe(III) or Fe(II) in these complexes was also confirmed both by EPR and Mössbauer spectra. Solid state reflectance spectra of **1**–**5** were found to be identical with those of aqueous absorption spectra, indicating that the structures of the compounds were intact even in water. Corresponding solid state reflectance data are shown in Table 3.

Table 2
Solution UV–Vis absorption data for iron–saccharide complexes as measured in H_2O, MeOH and *N*,*N*-DMF

Compd	λ (nm(ε, cm^{-1} mol^{-1})		
	H_2O	MeOH	*N*,*N*-DMF
1	290(6708), 350(3979), 450(27), 620(3)	239(19681), 370(5900), 480(313), 600(83)	270(564), 340(425), 490(202), 580(162)
2	285(1966), 369(5459), 470(535), 560(135)	233(19681), 370(5900), 480(313), 600(83)	269(504), 350(356), 490(150, 580(108)
3	292(1639), 360(820), 460(92), 570(18)	240(2732), 320(1423), 480(75), 580(32)	268(1509), 345(905), 480(259), 560(216)
4	294(8016), 340(3745), 450(58), 620(9)	239(38097), 320(20625), 460(115), 620(7)	269(1961), 360(1005), 470(76), 580(49)
5	285(4192), 369(1977), 470(161), 570(25)	242(13457), 330(304), 480(120), 560(38)	269(3749), 358(2852), 480(117), 580(59)
6	194(11232), 336(2477), 542(376)	227(18632), 330(6648), 560(517)	272(6056), 350(2913), 600(901)
7	191(14455), 260(6266), 330(1297), 570(169)	228(21395), 270(14397), 330(10928), 580(798)	275(17415), 340(7303), 600(999)

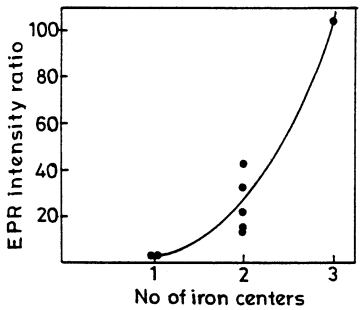

Fig. 2. Plot of EPR intensity ratio of the interactive to the isolated iron centers (I_{int}/I_{iso}) vs. number of iron-centers present in iron–saccharide complexes.

3.4. EPR spectra

EPR spectra of **1**–**7** exhibited a sharp signal corresponding to an isolated iron center (*iso*) and a broad signal corresponding to interacting iron centers (*int*) similar to those reported for **8**–**15**. Intensity ratios of these two signals (I_{int}/I_{iso}) in all the iron–saccharide complexes exhibited an interesting correlation with respect to the number of iron centers present in each complex, as shown in Fig. 2. Due to the non-linear behavior of this plot, the correlation cannot be used as a key for studying the nuclearity of all iron–saccharide complexes having greater numbers of metal centers; however, it was found suitable for the complexes synthesized with saccharides as reported by us. This is in agreement with the fact that the area of the *iso* peak becomes very small and overlaps with the *int* signal in the case of complexes having greater numbers of iron centers and, hence, its area cannot be measured accurately.

3.5. Mössbauer spectra

Mössbauer data shown in Table 4 are indicative of the presence of high-spin Fe(III) in all the complexes, and in addition, a high-spin Fe(II) center was found only in the cases of complexes **6** and **7**. Mössbauer spectra of complexes **2, 3,** and **5** can be fitted with a single, broad, quadrupole doublet with isomer shift values and quadrupole splitting parameters indicative of the presence of a high-spin ferric center with a distorted octahedral oxygen ligand environment created by the saccharide molecules. Similar high-spin Fe(III) Mössbauer characteristics were observed even in the cases of complexes **8**–**15**. In addition to the presence of the high-spin ferric center, complexes **6** and **7** have exhibited a high-spin ferrous center in a highly distorted octahedral environment (Table 4). Low temperature measurements carried out at 77°K in complexes **6** and **7** have suggested the presence of both Fe(II) and Fe(III) centers. Thus, the spectra of **6** and **7** could be fitted with two sets of quadrupole doublets. Based on these data, the ratio of Fe(II) to Fe(III) was found to be 1:1 for **6** and 1:2 for **7**. These ratios have also been confirmed based on chemical analysis.

3.6. Magnetic measurements

The effective magnetic moment value for **3** was found to be 5.6 BM at 298 K, a value that is comparable with that expected for mononuclear, high-spin Fe(III) complexes. On the other hand, low values of effective magnetic moments were found for **1**, **2**, **4** and **5** in the range 4.7–5.3 BM indicating the presence of some magnetic interaction between the iron centers. For **6** and **7**, variable temperature magnetic susceptibility measurements were performed in the temperature range 77–300 K and the data were fitted with standard equation, in order to obtain coupling constants. The coupling constants (J values) were found to be -30 and -40 cm^{-1}, respectively for **6** and **7**. These values are typical of the presence of antiferromagnetically coupled μ-hydroxo diiron centers of mixed valence compounds [12].

3.7. EXAFS and XANES spectra

The XANES spectra of complexes **8**–**12** exhibited a pre-edge structure possessing a 1s–3d transition char-

Table 4
Mössbauer data for iron–saccharide complexes

Compd	Temperature (K)	Fe(III)		Fe(II)	
		δ (mm s^{-1})	ΔE_q (mm s^{-1})	δ (mm s^{-1})	ΔE_q (mm s^{-1})
2	298	0.3176	0.8608	–	–
3	298	0.3074	0.9540	–	–
5	298	0.3124	0.8493	–	–
6	298	0.3315	0.8759	1.151	1.772
7	298	0.3018	0.8917	1.058	1.940
6	77	0.3918	0.8568	1.267	2.057
7	77	0.3530	0/8632	1.310	2.229

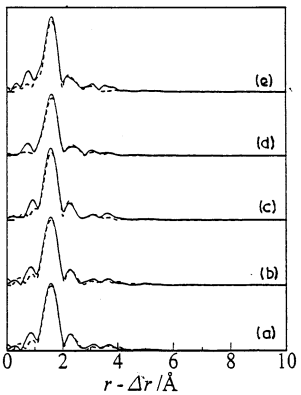

Fig. 3. Fourier transforms of iron–saccharide complexes; (a) **8**; (b) **9**; (c) **12**; (d) **11**; (e) **10**; as generated using EXAFS data. These Fourier transforms are not corrected for the phase shift.

acteristic of octahedral Fe(III) centers. While this transition is very sharp for the reference compound, $Fe(NO_3)_3{\cdot}9H_2O$, that possesses a $[Fe(OH_2)_6]^{3+}$ unit, the corresponding peak in the present complexes is found to be relatively broad indicating the presence of distorted octahedral Fe(III) centers.

Fig. 3 shows EXAFS spectra in their Fourier transform mode for complexes **8–12**. Since the phase shift is not corrected in the Fourier transformation, the peak position does not correspond to a true distance. The structural parameters of the solid iron–saccharide complexes **(8–12)** after applying phase shift correction are provided in Table 5. Fourier transforms generally exhibited (phase shift uncorrected) a predominant peak at around 1.60 Å, a small peak at 2.2 Å and in some cases small, broad peaks in the range 3–4 Å. The first peak is assigned to a direct bond between the Fe^{3+} and O atom of the saccharide moiety, and the second one to the interaction between the Fe^{3+} and C atom of the ligands, and the peaks observed in the range 3–4 Å to the third and fourth neighbor interactions from Fe^{3+}. For the dinuclear complexes, the Fe···Fe interaction also appears at around 2.2 Å (phase shift uncorrected), and agrees well with those distances reported in the literature based on crystal structures.

3.8. Solution stability and cyclic voltammetry

The solution stability of all the complexes has been studied in aqueous solution using cyclic voltammetry in the pH range 2–12. These studies have exhibited a linear correlation between the cathodic peak potentials (E_p^c) of the irreversible Fe(III) reduction and the pH of the solution for all the complexes. The slopes of these plots are 35, 26, 55, 44, 49, 17, and 30 mV pH unit^{-1}, respectively, for complexes **1–7** indicating that there seems to be only a simple proton-electron transfer associated with no hydrolytic cleavage. Thus, all the complexes were found to be stable in aqueous solution for several days without any recognizable precipitate formation. Such behavior is important for any complex, being considered as a potential dietary supplement. On the other hand, the complexes reported in the literature by Keypour et al. [13] have shown precipitation beyond pH 8.0. However, mixed valent complexes, **6** and **7,** have clearly indicated the conversion of Fe(II) to Fe(III) species in solution over a period of time in air, as studied by measuring the absorption spectra.

3.9. Putative bio-relevant studies

The iron–saccharide complexes have been subjected to diverse bio-relevant studies as reported in this paper.

3.10. Reductive release of iron(II)

The mobilization of ferritin has been studied both by the chelation of Fe(III) using appropriate chelators and by reductive dissolution of the ferrihydrite particles in the presence of simple reducing agents. However, the method of reductive release is particularly suited for interpreting in vivo iron release. Therefore, in order to establish the reductive release characteristics of these saccharide complexes, experiments were carried out using sodium dithionite, paramethylaminophenol or cysteine. Based on our experiments it is established that sodium dithionite acts as the most effective reducing agent when compared to the other two agents. Quanti-

Table 5
Data corresponding to the primary coordination around iron center(s) in the solid state iron–saccharide complexes based on EXAFS studies

Compd	Fe–O		Fe–C		Fe(1)–Fe(2)		Fe(1)–O(2)	Fe(2)–O(1)	*R*-factor
	r	$10^2\ \sigma^2$	*r*	$10^2\ \sigma^2$	*r*	$10^2\ \sigma^2$	*r*	$10^2\ \sigma^2$	
8	2.00(1)	0.48(8)	2.85(4)	0.8(6)	–	–	–	–	0.014
9	2.00(1)	0.47(9)	2.87(6)	1.2(9)	–	–	–	–	0.018
10	2.00(1)	0.62(14)	2.89(3)	0.11(40)	3.17(2)	0.50(21)	3.65(4)	0.27 fixed	0.0094
11	2.00(1)	0.59(15)	2.88(3)	0.15(41)	3.17(2)	0.51(22)	3.65(4)	0.27 fixed	0.0104
12	2.00(1)	0.63(15)	2.89(3)	0.13(40)	3.17(2)	0.48(20)	3.68(4)	0.27 fixed	0.0099

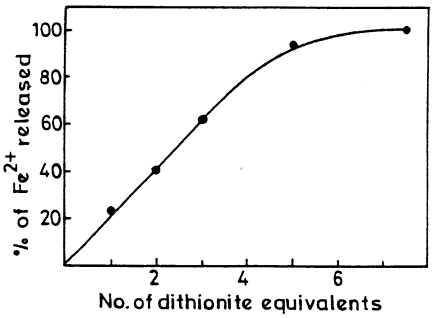

Fig. 4. Percentage of Fe^{2+} released as a function of number of sodium dithionite equivalents added. The percentages shown in the plot are the average values of the complexes **1**–**13**.

tative estimation of the Fe(II) released by the absorption spectra revealed that 5 equiv. of dithionite are required for total release of Fe(II) from the iron–saccharide complexes over a short period of time as shown in Fig. 4. However, the addition of three equivalents of sodium dithionite releases total iron from these complexes, but over a period of 24 h. Thus, the required concentration of dithionite is much less for the iron–saccharide complexes as compared to that for iron–dextran preparation [14].

The release of Fe(II) after the addition of 3 equiv. of sodium dithionite has been monitored by cyclic voltammetry as a function of time for all the complexes. Typical

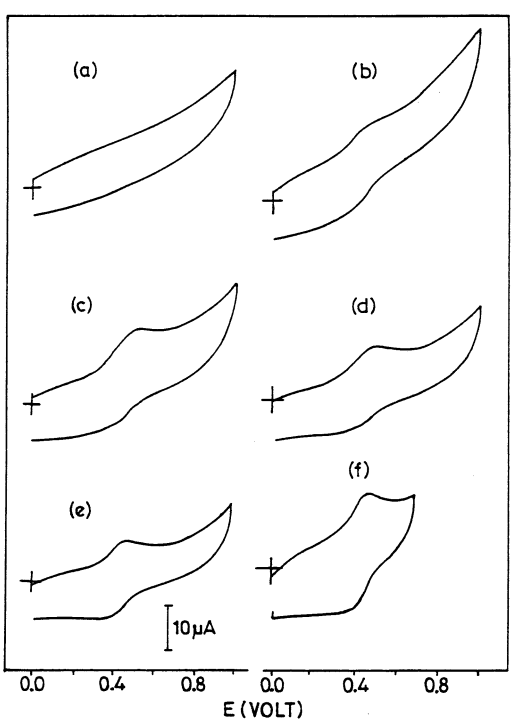

Fig. 5. Cyclic voltammograms of complex **1** as a function of time with the addition of 3 equiv. of sodium dithionite: (a) before the addition of the dithionite; (b) immediately after the addition; (c) 2.5 h after the addition; (d) 10 h after the addition; (e) 16 h after the addition; and (f) 24 h after the addition of sodium dithionite.

voltammograms are shown in Fig. 5 for complex **1**. The cyclic voltammetric study clearly revealed the presence of reduced species followed by release of Fe(II) as a function of time, and a complete release of Fe(II) in about 24 h. The corresponding voltammetric data are summarized in Table 6 for complexes **1**–**5** and **8**–**14**. In effect, the iron–saccharide complexes reported here have exhibited controlled reductive release properties which would be of importance if one were to use these iron–saccharide complexes as dietary nutrients. Thus, the complexes reported in this paper have exhibited better properties as compared to some of the iron-nutrient preparations reported earlier in the literature [15].

3.11. In vitro absorption studies

In order to understand the absorption of these complexes, an in vitro study was carried out with ^{59}Fe labeled complexes of **8** and **10** by measuring the transport of iron across the everted intestine as a function of time after incubation. The studies revealed that the absorption reaches a maximum within 30 min. Complex **8** showed higher efficiency of transport as compared to **10** as well as $FeCl_3$. Based on the concentration variation experiments of ATP it was understood that 5.0 mM ATP stimulates the absorption through intestinal membranes indicating that the iron absorption process under the present conditions is an active transport.

3.12. Protein synthesis

Protein synthesis was studied both in hemin-supplemented as well as in hemin-depleted rabbit reticulocyte lysates using five iron–saccharide complexes, **8**–**12**. In the case of hemin-supplemented reticulocyte lysate, the protein synthesis was not disturbed even in the presence of any iron–saccharide complexes. Removal of hemin from the lysate resulted in the complete loss of protein synthesis. However, when these hemin-deficient reticulocyte lysates were treated with iron–saccharide complexes (**8**–**12**), total protein synthesis was resumed. Thus, the iron–saccharide complexes, **8**–**12**, have exhibited the role that hemin generally plays in the protein synthesis, perhaps through supplementing the iron required. On the other hand, the vanadyl–saccharides have inhibited the protein synthesis both in the hemin-supplemented and hemin-deficient reticulocyte lysate under similar experimental conditions [9e]. Thus, the iron–saccharide complexes have exhibited behavior which contrasts that exhibited by vanadyl–saccharide complexes. This study has indicated the potential use of iron–saccharide complexes as nutrients. However, the present studies are not sufficient to deduce any mechanistic aspects regarding the role of iron–saccharide complexes.

Table 6
Cyclic voltammetric data for iron–saccharide complexes after the addition of 3 equiv. of sodium dithionite in aqueous solutions

Compd	Time (h)	E_p^a (mV)	E_p^c (mV)
1	0.0	465	346
	2.5	493	426
	6.5	474	421
	10.2	459	407
	16.0	465	392
	24.0	463	405
2	0.0	785, 486	363
	2.5	548	425
	6.5	483	411
	10.0	456	370
	16.0	463	400
	24.0	467	410
3	0.0	568	297
	3.0	704, 416	296
	7.0	668, 426	324
	10.5	628, 420	303
	16.0	415	304
	24.0	391	275
	31.0	378	280
4	0.0	656, 450	308
	3.0	656, 395	283
	7.0	595, 436	302
	10.5	782, 441	275
	16.0	419	281
	23.0	400	271
	32.0	415, 617	273
	45.0	413	292
5	0.0	440	275
	3.0	442	354
	7.0	458	415
	10.5	459	425
	16.0	462	420
	24.0	465	427
8	3.0	427	294
	6.5	428	278
	12.0	470	355
	16.0	490	420, 255
	22.0	485	389
9	1.0	790	89
	6.0	670	118
	17.0	494	290, 428
	30.0	491	394
10	4.4	440	318
	11.3	497	340
	17.3	491	363
	24.0	485	385
11	0.0	450	305
	3.0	440	318
	6.0	370	261
	10.0	436	315
12	0.0	440	350
	2.5	475	280
	6.5	470	290, 410
	10.5	462	300, 415
	16.0	460	405
	22.0	462	415
13	0.0	760	305
	4.0	385	265
	7.0	368	253
	9.0	380	294
	13.5	480	418
14	0.0	761	368
	2.5	529	384
	6.5	480	375
	10.0	475	415
	16.0	473	416
	24.0	472	416

3.13. DNA cleavage studies

The DNA cleavage properties of iron–saccharide complexes were evaluated in terms of the presence of percent of Form I (circular), II (nicked) and III (double nick or linear) in different experiments. The experiments included the interaction of the complex alone, complex + H_2O_2, and complex + H_2O_2 + mercaptoethanol with pUC-18 plasmid that is purified on a CsCl gradient. The results were interpreted only after comparison with appropriate controls. All the complexes except **2** and **15** have shown conversion of Form I to Form II in the range of about 16–60%. The DNA cleavage results are summarized in Table 7. However, the complete Form I was converted only when H_2O_2 was also used in the reaction. The addition of mercaptoethanol has not shown any influence on the DNA nicking property of the complexes. In the present experiments, after the addition of H_2O_2, Form III appeared to an extent of 5–40%. While complexes **2** and **15** have shown almost negligible effect, those of **3** and **6** have shown Form III even in the absence of H_2O_2 to an extent of 6 and 50%, respectively. Thus, the ascorbate complex **6** has been found to be most effective in DNA cleavage studies. As the concentration of the complexes used in these studies is rather large, the DNA cleaving properties of the complexes are not very important if these complexes were to be considered for use as nutrients.

3.14. Conclusions and correlations

The complexation of saccharides and/or ascorbic acid with iron has been well demonstrated based on simple FT-IR and UV–Vis studies. The *m*:*n* electrolyte behaviour of the iron–saccharide complexes has been very well correlated linearly with the observed ionic conductivity. However, the nuclearity of the complexes has been correlated non-linearly with the intensity ratios of the EPR peaks of the interactive center to that of the isolated center in all the complexes, **1** to **15**. The

Table 7
DNA cleavage data for pUC-18 plasmid under the influence of iron–saccharide

Compd = (a)	(a) + DNA + buffer = (b)			(b) + H_2O_2 = (c)			(c) + mercaptoethanol = (d)		
	Form I	Form II	Form III	Form I	Form II	Form III	Form I	Form II	Form III
None	62	38	–	53	47	–	46	54	–
2	68	32	–	49	51	–	49	51	–
3	35	59	6	–	85	15	–	80	20
6	–	50	50	smear [a]	30	30	smear	30	30
8	32	68	–	–	90	10	5	90	5
9	26	74	–	–	60	40	–	60	40
10	25	75	–	–	60	40	–	60	40
11	44	56	–	–	90	10	–	90	10
12	47	53	–	–	90	10	–	90	10
13	56	44	–	–	90	10	–	90	10
14	56	44	–	–	90	10	5	95	5
15	63	37	–	–	55	45	–	56	44

[a] Bands are diffused.

rather weak *anti*-ferromagnetic coupling observed with **6** and **7** has been interpreted as due to the presence of a hydroxo-bridge between the iron centers. While XANES studies have indicated the octahedral geometry around the metal center, EXAFS studies have delineated the binding of the saccharides through oxygen ligations. Mössbauer spectra have clearly demonstrated the presence of high-spin Fe(III) in a distorted octahedral environment in the complexes. The studies have further indicated the presence of high-spin Fe(II) in the cases of **6** and **7**. The cathodic peak potentials of all the complexes in solution exhibited a linear correlation with pH, though the slopes are different for different compounds. The studies are suggestive of the hydrolytic stability and robust nature of these complexes in aqueous solution over a wide range of pH.

The complexes have clearly exhibited a controlled mobilization and release of iron in the form of Fe(II) in the presence of reducing agent such as sodium dithionite. Complex **8** exhibited an active but efficient transport through everted sacs of rat intestines. While the vanadyl–saccharide complexes have shown protein synthesis inhibition in hemin-deficient rabbit reticulocyte lysate, the corresponding iron–saccharide complexes have exhibited resumption of total protein synthesis even in hemin-deficient reticulocyte lysate. The complexes have shown enhanced DNA cleavage properties in the presence of H_2O_2. Thus, the iron–saccharide complexes, reported by us, have high water solubility, good hydrolytic stability, controlled release of iron, efficient transport through membranes and can compliment the hemin deficiency in lysate and hence can act as good nutrients. However, further biological studies are required in order to assess the actual utility of these complexes for direct application as dietary iron supplements.

Acknowledgements

CPR is grateful to both the Department of Science and Technology (DST, Government of India) and Japan Society for Promotion of Science (JSPS, Government of Japan) for the award of a DST-JSPS research fellowship. CPR acknowledges the financial support from CSIR, DST and BRNS. AS thanks DAE for the award of K.S. Krishnan fellowship.

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