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Binding of Metal Ions to Polysaccharides. II. The Binding of Metal Ions to Chondroitin Sulphate in Alkaline Media

SIJBE BALT, MARTINUS W. G. DE BOLSTER* and GESINA VISSER-LUIRINK**

Department of Inorganic Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

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At high pH, polyelectrolytes like chondroitin sulphate form soluble complexes in water with a number of di- and trivalent cations probably through binding at the carboxylate groups. This interaction was investigated by ligand-field and ESR spectroscopy, H^+ and Cu^{2+} potentiometry, and viscometry.

Introduction

The glycosaminoglucuronan (or mucopolysaccharide) chondroitin sulphate‡ (Fig. 1) is a polyelectrolyte, which occurs in bone bound to a non-collagenous protein [1]. ChS plays a role in the transformation of cartilage to bone, in which calcium binding to this polyelectrolyte might be important [2].

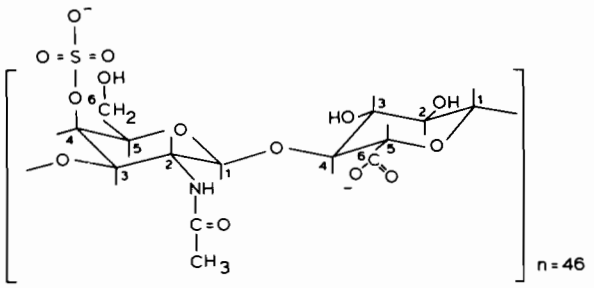

Fig. 1. Structure of chondroitin sulphate A. In chondroitin sulphate C the sulphate group is present in the 6-position and n = 69.

A number of investigations into the metal ion binding properties of this and other glycosaminoglucuronans have been carried out, but there is no agreement about the place and mode of binding (atmospheric or site) [3–5].

In addition to the charged carboxylate and sulphate groups, chondroitin sulphate contains an acetamido group. Uncharged polysaccharides containing an acetamido group, like the naturally occurring chitin (and its de-acetylated derivative chitosan), have been proposed as purifiers of water because they bind metal ions [6]. How exactly the binding takes place is not known; it has been suggested that the nitrogen atom is involved in the coordination [7]. In order to obtain a better insight into these phenomena we have started an investigation into the binding of metal ions to polysaccharides. Because of its high solubility in water, we started with chondroitin sulphate.

The first part of this series described polyelectrolyte catalysis by chondroitin sulphate [8]. The present paper reports on the coordination of metal ions to chondroitin sulphate and model compounds in alkaline medium by means of potentiometric, spectroscopic and viscosimetric techniques. Later parts of this series will report on interactions with metal ions in neutral media.

Experimental

Materials

Chondroitin sulphates were obtained from Sigma as disodium salts: ChSA from whale cartilage, ChSC from shark cartilage. The hygroscopic materials were stored over P_2O_5 *in vacuo*.

The weight averaged molecular weights ($\bar{M}_w$) were estimated by viscometry as 2.9×10^4 for ChSA [9] and 4.3×10^4 for ChSC [5]. The number averaged molecular weight ($\bar{M}_n$) was estimated as $\bar{M}_n = 0.8\ \bar{M}_w$ [10] giving $n_A = 46$ and $n_C = 69$. ^{13}C-NMR spectroscopy confirmed observations [11, 12] that ChSA contains about 15% sulphate in the 6-position and ChSC about 30% sulphate in the 4-position, most likely in the form of a co-polymer. Desulphation of ChSC to chondroitin (further indicated as Ch) was accomplished with the procedure of Kantor and Schubert [13].

*Author to whom correspondence should be addressed.
**Present address: Department of Chemical Technology, University of Amsterdam, Plantage Muidergracht 30, 1018 TV Amsterdam, The Netherlands.

‡The abbreviation ChS will be used. The systematic name for ChSA is: (1→4)-O-β-D-glucopyranosylic acid, (1→3)-2-acetamido-2-deoxy-β-D-galactopyranose-4-sulphate (or-6-sulphate for ChSC).

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Polyacrylic acid (PAA), with molecular weight stated as 90000, was obtained in the form of a concentrated aqueous solution from Aldrich. Small amounts of insoluble material were filtered off and the equivalent concentration of PAA was determined in a nitrogen filled glove box by titration with sodium hydroxide.

N-acetylglucosamine (NaG), the sodium salt of D-glucuronic acid (Gluc) and D-glucosamine as the HCl salt were obtained from Sigma. The free base D-glucosamine (Ga) was isolated using the procedure of Breuer [14]. Ytterbium nitrate was obtained from Alpha-Ventron. Tetramethylammonium perchlorate was precipitated from a solution of tetramethylammonium bromide (from Alpha-Ventron) by adding an excess of perchloric acid. 1,2-Bis(trimethylammonium)ethane perchlorate, $(CH_3)_3N^+CH_2CH_2N^+(CH_3)_3(ClO_4^-)_2$ (abbreviated as $N^{2+}(ClO_4^-)_2$), was prepared from N,N,N′,N′-tetramethyldiaminoethane (Merck) by methylation with methyl iodide and precipitation of the product with excess perchloric acid [15]. The pentaamminenitrocobalt(III) complex was prepared by the method of Mäueler [16]. The other metal perchlorates were Fluka analytical grade. Copper hydroxide was prepared according to Chao and Kearns [17] and bis(2,6 dimethyl-3,5-heptanedionato)copper(II) [Cu(dibm)$_2$] according to Graddon and Watton [18]. Ethylenediaminebis(2,4-pentanedionato)copper(II) [Cu(enacac)] was prepared as previously described [19].

Analyses

Copper and nickel were analyzed by complexometric titration with EDTA with murexide as indicator [20]. Sodium was determined gravimetrically as sodium zinc uranyl acetate, perchlorate as nitron perchlorate [20].

Spectroscopy

Spectra in the visible and UV region were recorded on a Beckman Acta M IV spectrophotometer. The standard conditions of the solutions were:

$[M^{n+}] = 2.5 \times 10^{-3}$ mol dm^{-3}

[ChSC] = 10^{-2} mol of disaccharide units dm^{-3}

$[OH^-] = 5.6 \times 10^{-3}$ mol dm^{-3}

$[N^{2+}] = 7.75 \times 10^{-2}$ mol dm^{-3}

ESR spectra were obtained with a Varian E3 spectrometer. The field was calibrated using diphenylpicrylhydrazyl (g = 2.00354). All the spectra were recorded as glasses at liquid nitrogen temperature, using solutions of about 2×10^{-2} mol dm^{-3} in copper(II). $^{13}C\{^1H\}$NMR spectra were recorded at 22.63 MHz on a Brucker WH90 in the Fourier Transform mode. IR spectra were obtained with a Perkin-Elmer 580B Infrared spectrophotometer; BaF_2 windows were used in the liquid cell.

Viscosimetric measurements

Viscosity was measured with the Lauda viscoboy, equipped with Ubbelohde-type viscometers (flow time of water 90.06 or 185.62 s at 298.2 ± 0.1 K) and an automatic timer. The solutions were those used for the ligand-field spectra.

Potentiometric measurements

Hydrogen-ion titrations were carried out in a nitrogen filled glove box in a thermostatted vessel at 298.1 ± 0.1 K using an Electrofact 7 G111 glass electrode and a double junction reference electrode consisting of an Ag-AgCl electrode and a salt bridge of 1 mol dm^{-3} $NaClO_4$, connected to an Electrofact 3600 mV-pH meter. Copper-ion titrations were carried out in the same solutions with a Philips IS-550 copper electrode, connected to the same mV meter [21].

Results

When sodium hydroxide is added to a solution containing copper(II) perchlorate and chondroitin sulphate no precipitate is formed, but the colour changes immediately from light blue to dark blue. This solution decomposes slowly; gradually a brown precipitate is formed. Similarly, polyacrylate (PA^-) keeps Cu^{2+} in solution at high pH. Because PA^- has no hydroxyl groups, this is a strong indication that deprotonation of hydroxyl groups of the polysaccharide ligand is not the cause of the phenomenon observed. Chondroitin sulphate, unlike glucose, does not reduce Cu^{2+} to Cu^+: when 2,9-dimethyl-1,10-phenanthroline is added the characteristic red colour of the copper(I) complex does not appear.

Upon addition of sodium hydroxide to a solution of nickel perchlorate and chondroitin sulphate, the colour changes slowly from green to yellow-green. After a few hours this reaction is complete, but the decomposition has then already started. Addition of a solution of chondroitin sulphate (brought to a high pH) to a solution of either copper(II) or nickel(II) also retains the metal ions in solution. However, a solution of chondroitin sulphate does not clear up a suspension of the metal hydroxides. It is possible to precipitate the complexes formed at high pH through addition of ethanol. The analytical results suggest the composition $M(ChS)_2(OH)_2Na_4$. The analytical composition of the copper complex was found as 5.69% Cu^{2+}, 8.39% Na^+ and 0% ClO_4^- (theoretically: 5.76% Cu^{2+}, 8.35% Na^+ and no ClO_4^-). For $Ni(ChS)_2(OH)_2Na_4$ these figures were: analysis 5.52% Ni^{2+}, 8.65% Na^+ and 0% ClO_4^- (theoretically: 5.35% Ni^{2+}, 8.38% Na^+ and no ClO_4^-).

We also investigated the reactions of chondroitin sulphate with other cations, in order to determine whether ChS could also keep other metal ions in

solution, that would otherwise precipitate as the hydroxide. In this connection we studied Mn^{2+}, Fe^{2+}, Co^{2+}, Zn^{2+}, Pb^{2+}, Al^{3+}, Cr^{3+}, Fe^{3+}, Yb^{3+}, Eu^{3+} and Pr^{3+}. In all cases no hydroxide precipitate was formed on addition of base when ChS was present, although the colour change (if observed) of the solutions indicated the formation of the hydroxide. An example of this is the course of the reaction of cobalt(II) with sodium hydroxide in the presence or in the absence of chondroitin sulphate. The colour of both reaction mixtures changes within one hour from blue via green to yellow. Also the change in pH upon hydroxide addition is the same with or without chondroitin sulphate.

In neutral medium Fe^{3+} forms a precipitate with chondroitin sulphate. Upon addition of hydroxide this precipitate dissolves. We used this reaction to get an indication, whether the action of OH^- is specific or a general anion effect operates. Upon addition of Cl^- instead of OH^-, the precipitate does not dissolve. This indicates strongly that OH^- indeed plays a specific role in this reaction.

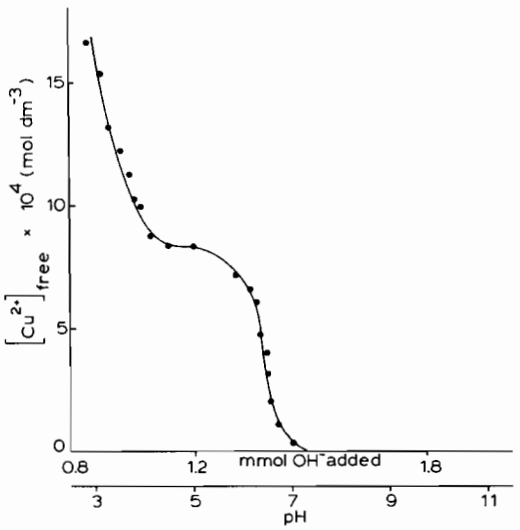

Fig. 2. Dependence of the concentration of free Cu^{2+} ions in a solution of ca 10^{-2} mol dm^{-3} ChS^{2-} on the amount of mmol OH^- added and the pH. Potentiometric titration of an aqueous solution (60 ml) of 0.3 mmol Cu^{2+}, 0.6 mmol ChS^{2-} and 1.2 mmol H^+, with OH^-.

Potentiometric Measurements

a) In Fig. 2 the titration of an acidified aqueous solution (60 ml) of 0.3 mmol Cu^{2+} in the presence of 0.6 mmol ChS^{2-} and 1.2 mmol H^+ with NaOH is shown. At low pH H^+ ions displace Cu^{2+} ions from ChS^{2-}. When the added 1.2 mmol H^+ has been neutralized, about 80% of the Cu^{2+} ions are bound by ChS. Upon addition of more hydroxide a slight turbidity is seen, which disappears quickly. The concentration of Cu^{2+} decreases to a very low value and the pH increases sharply after the equivalence point at two OH^- (1.8 mmol added). This equivalence point is independent of the ratio [ChS]/[Cu]. Copper hydroxide precipitates at high pH when the ratio [ChS]/[Cu] is smaller than one. Therefore we could not establish with a molar ratio curve [22] the number of chondroitin sulphate units bound to one Cu^{2+} ion. The titration curve does not show hysteresis, so the reaction is completely reversible. Chondroitin sulphate itself is neutral towards acid-base titration.

Titrations of the divalent cations (Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+} and Ba^{2+}) with hydroxide also show an equivalence point at two OH^-, except for Ca^{2+}, Ba^{2+} and $[Co(NH_3)_5NO_2]^{2+}$, but also without ChS these latter ions do not bind hydroxide. The investigated trivalent metal ions bind three hydroxide ions in the presence of ChS at high pH.

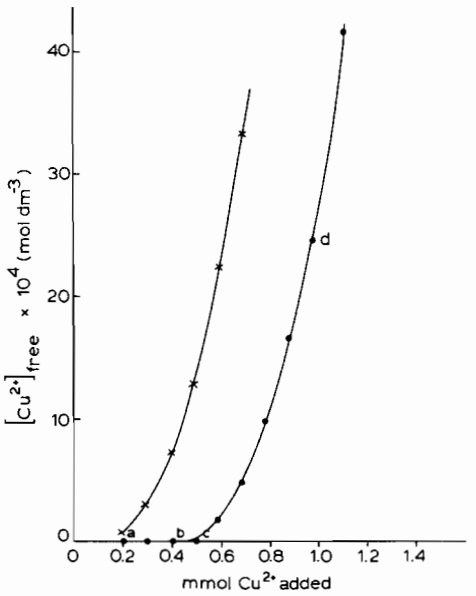

Fig. 3. Dependence of the concentration of free Cu^{2+} ions in a solution of ca 10^{-2} mol dm^{-3} ChS^{2-} on the amount of mmol Cu^{2+} added. · Potentiometric titration of an aqueous solution (60 ml) of 0.6 mmol ChS^{2-} and 0.67 mmol OH^-, with Cu^{2+}. x Potentiometric titration of an aqueous solution (60 ml) of 0.6 mmol ChS^{2-} with Cu^{2+}.
[a] pH = 11.9 [b] pH = 11.1 [c] pH = 7.1 [d] pH = 6.0

b) In Fig. 3 the titrations of chondroitin sulphate with copper(II) in basic and neutral medium are compared. It is difficult to measure the concentration of Cu^{2+} in basic medium [23]. Nevertheless it is clear that at high pH, only after addition of more Cu^{2+} than $[Cu^{2+}]/[OH^-] = ½$, free Cu^{2+} is present; then the pH decreases sharply. The difference between alkaline and neutral medium in the amount of copper(II) added to obtain the same free Cu^{2+} concentration is 0.34 mmol Cu^{2+} (equal to half the amount of alkali

TABLE I. ESR and Ligand-Field Spectral Data of Various Copper(II) Compounds.

Complex	$g_{\parallel}$ [a]	$A_{\parallel} \times 10^4$ (cm^{-1}) [b]	$g_{\perp}$ [c]	band maximum (kK) [d]
$[Cu(ChSA)_2(OH)_2]^{4-}$	2.25	197	2.06	15.1
$[Cu(ChSC)_2(OH)_2]^{4-}$	2.25	199	2.06	15.1
$[Cu(Ch)_2(OH)_2]^{2-}$	2.25	199	2.06	15.1
$[Cu(ChSA)_2(OH)_2]Na_2$ [e]	2.25	197	2.06	14.9
$[Cu(OH)_4]^{2-}$	2.25	193	2.06 [g]	15.0
$[Cu(dibm)_2]$ [f]	2.25	193	2.06 [h]	15.1
[Cu(enacac)]	2.18	219	2.01	18.4

[a] Estimated error ±0.01. [b] Estimated error $\pm 2.10^{-4}$ cm^{-1}. [c] Estimated error ±0.03. [d] Estimated error ±0.1 kK. [e] Solid. [f] In toluene. [g] Reference 17. [h] Reference 24.

added). This difference remains almost constant during the titration. This means that the binding of extra copper(II) is not significantly influenced by $Cu(OH)_2$ already bound.

ESR and Ligand-Field Spectra

In Table I the ESR and ligand-field spectral data of solutions of copper(II) in the presence of chondroitin sulphate and chondroitin are compared with spectra of square planar complexes with (charged) oxygen donor atoms ($Cu(OH)_4^{2-}$ [17] and $Cu(dibm)_2$ [24]) and a complex with oxygen and nitrogen coordination [Cu(enacac)]. Only in the case of the enacac complex was nitrogen superhyperfine splitting observed (15×10^{-4} cm^{-1}). Further, in the ESR spectra no signal at $\Delta m = 2$ was found. From these results it can be concluded that in the ChS and Ch compounds the coordination around the metal ions is square planar [25] and that no copper(II) dimer is formed [26]. It is obvious that the ligands coordinate only *via* oxygen atoms. Therefore the nitrogen atom of the N-acetylgroup is not involved in the coordination.

We also recorded ESR and ligand-field spectra of copper(II) complexes of polyacrylate and of monosaccharides with an acetamido group (N-acetylglucosamine), a carboxylate group (glucuronate) and an amine group (glucosamine) at high pH. Only in the case of glucosamine was evidence found for nitrogen coordination. The spectra of the other compounds were identical with that of the copper(II) chondroitin sulphate system.

In Table II are listed the spectral data in the visible and UV region of solutions of some other metal ions and ChS at high pH. The spectrum of Fe^{2+} indicates an octahedral coordination [25]. The spectrum of Co^{2+} changes completely on going from alkaline to neutral medium [21]; in the former it resembles the spectrum of $[Co(OH)_4]^{2-}$, where Co^{2+} is tetrahedrally surrounded [27]. Ni^{2+} and Cr^{3+} are surrounded by an octahedron; the spectra changed only slightly with respect to those in neutral medium and those of the aqua complexes. Only in the cases of Ni^{2+} and Cr^{3+} are the final complexes octahedra, possibly connected with the fact that these reactions are slow.

TABLE II. Ligand-Field Data of Solutions of Various Metal Ions in the Presence of Chondroitin Sulphate at High pH. [a]

Metal ion	Band maxima (kK) and extinction coefficient ϵ (dm^3 mol^{-1} cm^{-1})		
Fe^{2+}	14.9(30)		
Co^{2+}	15.6(52)	16.9(42)	
Ni^{2+}	13.5(3)	15.0(4)	25.6(14)
Hg^{2+}	35.7(227)		
Cr^{3+}	17.2(23)	24.9 sh	

[a] At standard conditions (see experimental part).

Viscosities of Solutions of Chondroitin Sulphate with a Number of Metal Ions at High pH

In Table III the viscosities are listed of solutions of various metal salts in the presence of chondroitin sulphate at standard conditions.

It is striking that the value of the relative viscosity η_{rel} ($\eta_{solution}/\eta_{solvent}$) of solutions with metal ions at high pH is higher than or equal to η_{rel} of the solution with only N^{2+}. Without hydroxide, all relative viscosities are lower than that of a reference solution (containing ChS and N^{2+}), due to screening of the negative charges on the polyelectrolyte [28]. In general the increase in the viscosities of the trivalent metal ion solutions is more pronounced than that of the divalent metal ion ones. The latter viscosities increase according to the Irving-Williams series [29], just as in neutral medium (with the exception of cobalt(II)).

For the system with copper(II) we measured the viscosity as a function of the Cu^{2+} concentration, while the other reagents were at standard conditions (Table IV). The relative viscosity shows a maximum at $[Cu^{2+}]/[OH^-] = 1/2$; at the other ratios η_{rel} is smaller, probably due to an excess of Cu^{2+} or Na^+.

There are indications that in cartilage ChS chains interact [30, 31]. Therefore we tried to obtain some information about a possible shear-stress belonging

TABLE III. Relative Viscosities of Solutions of Various Cations in the Presence of Chondroitin Sulphate at High pH.[a]

Cation	η_{rel}[b,c]	η_{rel}[c]	pH[d]
N^{2+} [e]	1.76	1.73	11.2
Ca^{2+}	1.74	1.74	11.2
Mg^{2+}	1.74	1.75	9.2
Mn^{2+}	1.74	1.83	9.2
Fe^{2+} [f]	–	1.83	8.5
Co^{2+}	1.74	1.92	9.2
Ni^{2+}	1.74	1.87	9.5
Cu^{2+}	1.71	2.08	8.2
Zn^{2+}	1.74	1.76	9.0
Hg^{2+}	1.74	1.74	8.0
Pb^{2+}	1.75	2.01	9.2
Ba^{2+}	1.74	1.76	11.7
$[Co(NH_3)_5NO_2]^{2+}$	1.77	1.74	11.5
$N(CH_3)_4^+$	1.76	1.74	9.7
Al^{3+}	1.73	1.82	6.0
Cr^{3+}	1.67	1.73	6.0
Fe^{3+}	1.77	1.94	6.0
Yb^{3+}	1.76	2.46	7.5
Eu^{3+}	1.66	2.67	8.2
Pr^{3+}	1.72	2.68	8.7

[a]At standard conditions (see experimental part). [b]Without hydroxide, pH < 6.5. [c]Estimated error ±0.005. [d]Estimated error ±0.2. [e]N^{2+} = 1,2-bis(trimethylammonium)-ethane: $(CH_3)_3\overset{+}{N}CH_2CH_2\overset{+}{N}(CH_3)_3$. [f]On iron powder.

TABLE IV. Viscosity Measurements of Solutions of Various Concentrations of Copper(II) in the Presence of Chondroitin Sulphate at High pH.[a]

$[Cu^{2+}]/[ChS^{2-}]/[OH^-]$ [b]			pH[c]	η_{rel}[d]
4	1	1/2	[e]	[e]
2	1	1/2	[e]	[e]
1	1	1/2	5.7	1.73
1/2	1	1/2	6.3	1.79
1/4	1	1/2	8.0	2.08
1/8	1	1/2	10.3	1.97
0	1	1/2	11.5	1.73

[a]At standard conditions (see experimental part). [b]Concentration ratio. [c]Estimated error ±0.2. [d]Estimated error ±0.005. [e]Precipitate formed.

to this interaction for our compounds. We measured the viscosities of a solution of $1Cu^{2+}/2ChS^{2-}/2OH^-$ and of a solution of $1Yb^{3+}/3ChS^{2-}/3OH^-$ with a concentration of 10^{-3} to 10^{-2} mol dm^{-3} ChS with two different Ubbelohde viscometers: one having a flow time of water of 185.62 seconds, the other of 90.06 seconds. The viscosities were found to be independent of the flow time. However, this does not exclude the presence of shear-stresses; the range of flow times might not have been large enough to show differences.

^{13}C-NMR Spectra

Recently, a complete assignment of the ^{13}C-NMR shifts of chondroitin sulphate has been published [12]. We tried to get some information from ^{13}C-NMR about the structure of the complexes formed. With Cu^{2+} as well as with Yb^{3+} the chemical shifts at high pH were found to have the same values as uncomplexed ChS. Hiraoki *et al.* mention a similar phenomenon in the case of the Cu(II)-poly-D-glutamate system at pH 9 [32]. They suggest that only the uncomplexed part of the ligand is measured.

Saitô *et al.* [33] found that the ^{13}C-NMR signals of a gel of a (1→3)-β-D-glucan were very broad and less intense than those of a solution of a low molecular weight fraction. Because the viscosity of our systems increases considerably compared to the complexes in neutral medium, we assume that the chondroitin sulphate complexes form a gel at high pH, and that we indeed measure only the uncomplexed ligand.

^{1}H-NMR Spectra

It was not possible to obtain well-separated peaks in the OH absorption region in the presence of copper(II). However, the chemical shift of the NH group was observed in the usual region. It did not vary with pH and showed no change upon coordination. It can therefore be concluded that the NH group is not deprotonated and that the N-acetyl group is not involved in the coordination.

Reactions of Related Monosaccharide Compounds

The reactions of copper(II) and nickel(II) with N-acetylglucosamine, sodium glucuronate and glucosamine were also investigated in order to establish at which functional group coordination is preferred. It turned out that comparison with these monosaccharides is not possible, because the C_1–OH group (blocked in ChS) is involved in the coordination. However, from IR spectra of N-acetylglucosamine in deuterium oxide it is clear that the acetamido group does not coordinate to copper(II) at high pH. Further results will be published elsewhere [34].

Discussion

From the results of the ligand-field and ESR measurements we concluded that in the complexes formed with ChS at high pH the metal ions are surrounded by oxygen donor atoms. pH measurements indicated a $2OH^-/1\ M^{2+}$ and a $3OH^-/1\ M^{3+}$ ratio.

We have found that in neutral medium, metal ions are bound to carboxylate groups of ChS. There seems

to be only a small electrostatic contribution from the sulphate group [21]. Moreover we found no difference in behaviour for ChSA, ChSC and Ch with copper(II) at high pH. It seems possible that the way in which metal ions are bound to the polyelectrolyte in neutral medium prevents the formation of polymeric hydroxides when base is added. This proposition is schematically represented in Fig. 4. This structure resembles the one postulated by Namoto *et al.* [35] for the copper complex of mugineic acid. This ligand is, as well as ChS, capable of effectively solubilizing $Fe(OH)_3$. Also the fact that $Cu(OH)_2$ is bound without influence on the binding of more copper ions to the polyelectrolyte is in agreement with this model. A neutral species like $Cu(OH)_2$ has no influence on the charge of the polyelectrolyte. More copper ions can then be bound by $[Cu(ChS)_2(OH)_2]^{4-}$ *via* uncomplexed oxygen atoms.

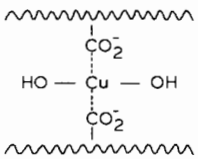

Fig. 4. Proposed coordination of $Cu(OH)_2$ to ChS.

The increase of viscosity of solutions of chondroitin sulphate complexes at high pH indicates the formation of a gel structure with junction zones. Therefore we assume that at high pH not just two chains (as in neutral medium [36]) but more interact. In the literature the assumption is frequently found that at high pH polysaccharides are deprotonated. For instance, Welti *et al.* [37] assume that hydroxyl groups of chondroitin sulphate and hyaluronate are ionized at high pH. From small shifts in the ^{1}H-NMR spectrum of hyaluronate they conclude that specific attraction between the 2-OH group and the acetamido group changes to repulsion in basic medium. They also mention another indication for this specific intramolecular association, *viz.* the increase of viscosity at high ionic strength. However, our experiments show that this is only the case if the added electrolyte is a salt of a cation that can be precipitated as the hydroxide (see Table III).

In the literature we could only find one example of a comparable reaction of a polyelectrolyte with copper(II) at high pH. Leyte [38] investigated extensively the interaction of copper(II) with polymethacrylic acid in neutral medium. For ratios [PMA]/[Cu] = 2 and higher he noticed that "after continued addition of alkali the solution becomes clear again at pH = 9. The dissolution always starts after the point of equivalence with $Cu(NO_3)_2$ has been reached". Leyte assumed that after binding of 1 Cu per 2 carboxylate groups a dissociation of the remaining carboxylic acid groups is responsible for this observation. However, in our case definitely no undissociated COOH groups are present.

In our opinion the resemblance of the reaction of metal ions with hydroxide with or without chondroitin sulphate and the similarity with the reaction with polyacrylate indicate a coordination of hydroxide to the metal ion.

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