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Binding of Metal Ions to Polysaccharides. VI. Spectroscopic and Potentiometric Studies of the Binding of Copper(II) and Nickel(II) to some Monosaccharide Units in Acidic, Neutral and Alkaline Aqueous Media

ELEONORA B. V. APPELMAN-LIPPENS, MARTINUS W. G. DE BOLSTER*, DOUWE N. TIEMERSMA and GESINA VISSER-LUIRINK**

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

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Abstract

Binding of Cu^{2+} and Ni^{2+} to glucosamine, N-acetylglucosamine and other derivatives of glucose was investigated in acidic, neutral and alkaline aqueous media using H^+ and Cu^{2+} potentiometry and ligand-field and ESR spectroscopy. In neutral medium, site binding with copper(II) and nickel(II) occurs when the monosaccharide possesses a potentially coordinating amine or charged group not attached to C-1. At high pH, a coordination entity is only formed if the C-1 hydroxyl group can be deprotonated and other stabilizing groups are present. The role of groups attached to C-1 reflects the different behaviour of monosaccharides compared with polysaccharides.

Introduction

Previously, in this series [1–5], investigation into the binding of cations to glycosaminoglycans such as chondroitin sulphate, chondroitin, chitin and chitosan has been described. In support of these studies on polysaccharides, it was necessary to study the interaction of metal ions with the monosaccharide units. A different behaviour may be expected because of the possible involvement in the monosaccharides of groups attached to C-1. In the literature, some controversies regarding the interaction of metal ions with monosaccharides can be found. Tamura and Miyazaki [6], for instance, explored the interaction between copper(II) and glucosamine and concluded that a 1:1 complex was formed. Inaki *et al.* [7], however, suggested a 1:2 ratio and deprotonation of the C-3 hydroxyl group at high pH. It seemed, therefore, worthwhile to study the interaction between metal ions (Cu^{2+} and Ni^{2+}) and glucosamine (the monosaccharide unit of chitosan) and N-acetylglucosamine (present in chitin and chondroitin) more fully. The present paper describes such a study. For comparison, the interaction between Cu^{2+} and glucuronate, glucose, glucose 1-phosphate, glucose 6-phosphate, tetramethylglucose, glucosamine pentaacetate and N-acetylcyclohexylamine was also studied.

Experimental

The metal perchlorates, N-acetylglucosamine (NaG), sodium glucuronate (NaGluc), the HCl salt of glucosamine, glucose, tetramethylglucose, glucose 1-phosphate and glucose 6-phosphate used were commercial products (Fluka and Sigma). The free base glucosamine (Ga) was isolated using the Breuer [8] procedure. Glucosamine pentaacetate was prepared by the method of Whitaker *et al.* [9] and N-acetylcyclohexylamine as described [10]. Analyses, potentiometric and spectroscopic measurements were undertaken as described previously [1–5].

Results

When copper(II) perchlorate is added to a solution containing glucosamine or glucuronate, a complex is formed. In the case of N-acetylglucosamine no reaction takes place. When sodium hydroxide is added to the solutions, no precipitate is formed but the colour changes immediately to dark blue. The solutions decompose slowly; gradually a brown precipitate is formed. To arrive at an indication of the role of substituents in the coordination, a number of monosaccharides was investigated. Glucose and glucose 6-phosphate do not react with Cu^{2+} in a neutral medium; at high pH the metal ion is retained in solution.

*Author to whom correspondence should be addressed.
**Present address: Gist-Brocades N.V., Wateringseweg 1, 2600 MA Delft, The Netherlands.

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Tetramethylglucose, glucose 1-phosphate, glucosamine pentaacetate and N-acetylcyclohexylamine do not react with Cu^{2+} at any pH. It is possible to precipitate some of the complexes formed at high pH by means of the addition of an excess of ethanol. *Anal.* Found for $Cu(H_{-1}Ga)_2 \cdot H_2O$: Cu, 14.4; C, 32.5; H, 5.86%. Calc. Cu, 14.51; C, 32.92; H, 5.98%. Found for $Na_2Cu(H_{-1}NaG)_2(OH)_2 \cdot 0.2Cu(OH)_2$: Cu, 12.6; Na, 7.59%. Calc. Cu, 12.67, Na, 7.65%. In the case of glucosamine, a nickel complex was also prepared. Found for $Ni(H_{-1}Ga)_2 \cdot 2H_2O$: Ni, 13.0, C, 32.3; H, 5.98; N, 6.14%. Calc. Ni, 13.01, C, 31.95, H, 6.27, N, 6.21.

Potentiometric Measurements

(a) Acid-base titrations of the monosaccharides

For a general monosaccharide (Mon) the dissociation constants K_i can be defined as:

$$K_1 = \frac{[\text{Mon}]\,[H^+]}{[\text{HMon}]} \text{ and } K_2 = \frac{[H_{-1}\text{Mon}]\,[H^+]}{[\text{Mon}]}$$

In the case of glucosamine, for instance, H_{-1}Mon denotes glucosamine with a deprotonated hydroxyl group on C-1 and HMon denotes glucosamine with a protonated amine group. The monosaccharide can be present in the α or in the β form.

For glucosamine, the values found for $pK_1 = 7.8$ and $pK_2 = 12.3$ (at an ionic strength, I, of 1 mol dm^{-3}) by Neuberger and Fletcher [11] were confirmed. N-acetylglucosamine does not react with acid. For the reaction with base, a value of $pK_2 = 12.3$ ($I = 1$) was found. The similarity of the pK_2 values strongly suggests that the same group is involved in the deprotonation reaction. For glucuronate, the value of $pK_2 = 12.1$ found by Makridou *et al.* [12] was also confirmed.

(b) Acid-base titrations of the monosaccharides in the presence of Cu^{2+}

For glucosamine it was found that 0.5 mmol Cu^{2+} and 1 mmol Ga bind 1 mmol OH^- and that 0.25 mmol Cu^{2+} and 1 mmol Ga bind 0.5 mmol OH^-. During the titration copper(II) hydroxide precipitates and dissolves when the ratio Ga/Cu^{2+} is larger than 2. In the case of nickel(II) a similar behaviour was found; however, the complexes are less stable.

For N-acetylglucosamine and glucose it was found that 0.5 mmol Cu^{2+} and 1 mmol ligand bind 2 mmol OH^-. Tetramethylglucose, glucose 1-phosphate, glucosamine pentaacetate and N-acetylcyclohexylamine do not form a complex with Cu^{2+} in neutral or alkaline medium. Glucuronate and glucose 6-phosphate react in alkaline medium in the ratio Cu^{2+}:L:$OH^- = 1:2:2$.

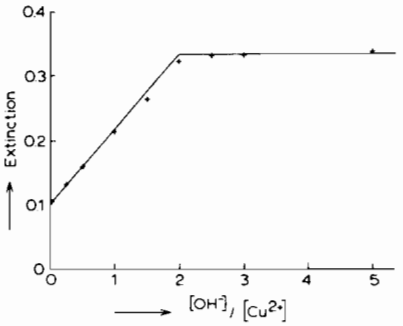

Fig. 1. Molar ratio curve of solutions of 0.01 M $Cu(ClO_4)_2$, 0.02 M glucosamine and NaOH; extinctions at 18.2 kK.

TABLE I. Ligand-field Spectral Data for the Copper(II)–Glucosamine System.

Ratio Cu^{2+}:Ga:OH^-	pH[a]	Band maximum[b] (kK)	Extinction coefficient[c] ($l\,mol^{-1}\,cm^{-1}$)
1:0:0	4.5	12.5	11.2
1:0.5:0	5.5	13.2	16.8
1:1:0	5.5	13.7	23.7
1:2:0	5.5	14.4	38.2
1:2.5:0	6.0	14.7	43.8
1:3:0	6.0	14.9	47.5
1:4:0	6.5	15.4	46.7
1:5:0	7.0	15.9	40.8
1:6:0	7.5	16.0	42.2
1:12:0	8.0	16.1	43.2
1:2:0.5	6.5	14.7	46.3
1:2:1	7.0	15.1	47.9
1:2:1.5	7.5	15.5	45.8
1:2:2	9.5	16.1	40.2
1:2:2.5	11.5	16.1	40.1
1:2:3	12.0	16.2	40.4
1:4:2	8.5	16.3	38.5
1:16:2	9.5	16.3	40.0

[a]Estimated error ± 0.2. [b]Estimated error ± 0.1 kK. [c]Estimated error ± 0.2 $l\,mol^{-1}\,cm^{-1}$.

Ligand-field Spectra

To determine the composition of the complexes, the well-known molar ratio method [13] was used. The ligand-field spectra of a series of solutions with a fixed metal concentration and various ligand or hydroxide concentrations were recorded. A typical molar ratio curve is shown in Fig. 1.

For the band maxima and extinction coefficients for the Cu^{2+}–glucosamine–(OH^-) system, see Table I. In a neutral medium, the molar ratio curve indicates the existence of a 1:2 complex. In alkaline medium compounds with the ratio Cu^{2+}:ligand:OH^- = 1:2:1 and 1:2:2 were found. For Ni^{2+} the results indicate the existence of a 1:2 complex in neutral and alkaline media. In the case of N-acetylglucos-

amine, the results are in accordance with the existence of a 1:2:4 copper complex in an alkaline medium. To establish the nature of the donor atoms in the copper glucosamine complexes in a neutral and alkaline medium, the method described by Kurganov and Davankov [14] was used. From spectra of a series of copper complexes, these authors estimated the average contribution of the ligands in the main plane (ν_i) and in the axial positions (ν_j) to the band maximum (ν_{calc}), which can then be calculated as:

$$\nu_{calc} = \sum_{i=1}^{4} \nu_i - \left(\sum_{j=1}^{2} \nu_j - \Delta \right).$$

In this formula, Δ represents a correction for possible steric hindrance of the axial groups. For the ligands of amine, water, carboxylate and hydroxide the contributions are (in kK) 5.40, 4.40, 4.85 and 4.50 for ν_i and 3.30, 2.50, 3.20 and 3.90 for ν_j.

For the copper–glucosamine system, comparison of calculated and observed band maxima indicate, in a neutral medium, the presence of complexes of composition $[Cu(Ga)_n(H_2O)_{6-n}]^{2+}$ ($n = 1, 2$) with glucosamine coordinating via the amine group. In alkaline medium, the existence of $[Cu(H_{-1}Ga)_2(H_2O)_2]$ is indicated with the ligand coordinating via the amine group and a deprotonated hydroxyl group. For the latter, the carboxylate contribution was used in the calculations and Δ was set to zero. It is noticed that these calculations do not exclude the possibility that OH^- directly coordinates to the metal ion and that the complex formed should be formulated as $[Cu(OH)_2(Ga)_2(H_2O)_2]$. In view of the results of the potentiometric measurements, this is, however, unlikely [1–5].

ESR Spectra

Table II shows the ESR spectral data of a number of copper(II) monosaccharide solutions. Comparison with published spectra of chondroitin sulphate and other systems [1, 4] demonstrates the following indications about coordination: only in the case of glucosamine, nitrogen atoms are involved in the coordination around the metal ion. For N-acetylglucosamine and glucuronate at high pH, the coordination around the metal ions is square planar. These ligands only coordinate via oxygen atoms and the nitrogen atom of the N-acetyl group, therefore, is not involved in the coordination. Because no signal at $\Delta m = 2$ was found in the spectra, copper(II) dimers were not formed.

TABLE II. ESR Spectral Data of Various Copper(II)–Monosaccharide Systems.

Ligand	Ratio Cu^{2+}:L:OH^-	$g_\parallel$[a]	$A_\parallel \times 10^4$ (cm^{-1})[b]	$g_\perp$[c]
Ga	1:2.5:2	2.23	207	2.07
	1:12:0	2.25	191	2.06
NaG	1:2.5:1	2.41	141	2.07
	1:2.5:4	2.25	190	2.07
Gluc	1:2:2	2.25	198	2.06
	1:2:0	2.40	149	2.06

[a]Estimated error ± 0.01. [b]Estimated error ±2 × 10^{-4} cm^{-1}. [c]Estimated error ± 0.03.

Discussion

The Metal–Glucosamine System

Glucosamine and its conjugate base form various complexes with copper(II), depending on the (relative) concentrations of metal and ligand and the pH. In aqueous solution, the following equilibria play a role (coordinated H_2O is omitted):

$$H_1Ga^+ \rightleftharpoons Ga + H^+$$

$$Ga + OH^- \rightleftharpoons H_{-1}Ga^- + H_2O$$

$$Cu^{2+} + Ga \rightleftharpoons Cu(Ga)^{2+}$$

$$Cu(Ga)^{2+} + Ga \rightleftharpoons Cu(Ga)_2^{2+}$$

$$Cu(Ga)^{2+} + OH^- \rightleftharpoons Cu(H_{-1}Ga)^+ + H_2O$$

$$Cu(Ga)_2^{2+} + OH^- \rightleftharpoons Cu(H_{-1}Ga)(Ga)^+ + H_2O$$

$$Cu(H_{-1}Ga)(Ga)^+ + OH^- \rightleftharpoons Cu(H_{-1}Ga)_2 + H_2O$$

$$Cu(H_{-1}Ga)^+ + OH^- + H_2O \longrightarrow Cu(OH)_2\downarrow + Ga \quad \text{(approximated)}$$

$$Cu(H_{-1}Ga)_2 + OH^- + H_2O \longrightarrow Cu(OH)_2\downarrow + Ga + H_{-1}Ga^- \quad \text{(approximated)}$$

In a solution of copper(II) perchlorate and glucosamine in a 1:1 ratio without extra base or acid, the 1:1 complex $Cu(Ga)^{2+}$ is formed. This complex is able to bind one hydroxide ion but is very susceptible to decomposition. Upon addition of more hydroxide, $Cu(OH)_2$ precipitates, as has been described by Tamura and Miyazaki [6]. When the concentration of glucosamine is larger than that of copper(II), the 1:2 complex $Cu(Ga)_2^{2+}$ is formed. This complex can react with hydroxide ions in discrete steps, resulting in the complexes $Cu(H_{-1}Ga)(Ga)^+$ and $Cu(H_{-1}Ga)_2$. These complexes are extremely susceptible to base hydrolysis.

Umezawa *et al.* [15] investigated the complex formation of a number of 1-O-methyl-α- and β-D-glucosamine compounds (by determining the optical rotation shifts) and concluded that complex formation only occurs for compounds that contain a

hydroxyl group next to an amine group. In the case of glucosamine, the hydroxyl groups on C-1 and C-3 would then be suitable. However, the band maximum in the ligand-field spectra of the copper(II)–glucosamine complexes can be calculated [14], assuming coordination by the amine group and water. Using a hydroxyl group instead of water in the calculations should hardly influence the ligand contribution but will influence the steric hindrance factor. We assume that, in the glucosamine complexes, coordination takes place via the amine group and water. In the complexes of deprotonated glucosamine ($H_{-1}Ga$), both the amine group and the deprotonated anomer hydroxyl group (on C-1) are involved. This is supported by the results of the ESR measurements [16].

Tamura and Miyazaki [6] assume the existence of only a 1:1 complex in solution. It is, however, hardly realistic to involve a coordinated hydroxide ion in the equilibria at low pH and to neglect the possibility of deprotonation of glucosamine. Yaku *et al.* [17] presented a molar ratio curve for the copper–glucosamine system which suggests the existence of a 1:4 complex. They do not mention the pH of the solutions neither the amount of hydroxide that was added. Furthermore, they mention that some precipitate was removed by filtration; this tends to suggest that their results are unreliable. Inaki *et al.* [7] recorded spectra of 1:10 solutions at various pH values. Their spectra are similar to those we obtained for 1:2 systems. They conclude that, at high pH, hydrolysis does occur with deprotonation of hydroxyl groups. They suggest that the hydroxyl group attached to C-3 is involved in this reaction. Because the hydroxyl group attached to C-1 is more acidic [11], this seems unlikely. Probably, these authors reach this erroneous conclusion because of their comparison with chitosan (the polymer of glucosamine) where the oxygen atom at C-1 is involved in the polymerization linkage.

In Table III, ligand-field spectral data for the copper(II) and nickel(II) glucosamine complexes are compared with literature data [18–21]. This comparison confirms the conclusion that glucosamine coordinates via the nitrogen atom and that no more than two glucosamine ligands are involved in the coordination. In the 1:2 copper(II) glucosamine complexes, the ligands are in *cis* positions.

The Copper–N-acetylglucosamine System

N-acetylglucosamine only forms a complex with copper(II) when hydroxide has been added. The composition of the complex, according to the molar ratio curves is $[Cu(H_{-1}NaG)_2(OH)_2]^{2-}$. From the ESR spectra it can be concluded that the coordination around the metal is square planar, that the ligands coordinate via oxygen atoms only and that no dimer is formed. Furthermore, four hydroxide ions are involved in the complex formation. Because NaG itself binds one OH^-, two hydroxide ions are directly bound to the metal ion. Infrared and ^{13}C NMR spectra indicate that the N-acetyl group is not involved in the reaction with base [5]. Therefore, we conclude that Cu^{2+} coordinates to OH^- and the deprotonated hydroxyl group on C-1. The copper–glucose system behaves similarly although the complexes so formed are less stable indicating that the N-acetyl group acts, somehow, as a stabilizing factor.

TABLE III. Ligand-field Spectral Data for Some Copper(II) and Nickel(II) Complexes.

Donor set (ligand)	Band maximum[a] (kK)	
	Cu(II)	Ni(II)
O_6	12.5	8.5
N_1O_5	13.4	9.1
N_2O_4	14.7[c]	9.6
N_3O_3	15.5	10.0
N_4O_2	16.9	10.5
$(glycinato\text{-}N,O)_2$[b]	15.8	9.8
Ga[b]	13.7	9.2
$(Ga)_2$[b]	14.7	9.5
$(H_{-1}Ga)_2$[b]	16.1	9.9

[a]Literature data from refs. 18–21. [b]Coordinated H_2O is omitted. [c]*Cis*-compound (*trans* at 16.7 kK).

The Metal–Glucuronate System

It has already been concluded from ^{13}C NMR spectra that the carboxylate group is involved in the coordination [3]. Also, it was established from pCu measurements that not more than two glucuronate ions are bound per copper ion [4]. When hydroxide is added, two OH^- ions are bound. It is assumed, therefore, that the hydroxyl group on C-1 is deprotonated and involved in the coordination (together with the carboxylate group). This is supported by the results of the ESR measurements. In the case of nickel(II), the complex formed is less stable.

Conclusion

In neutral medium, only glucosamine, glucuronate and glucose 6-phosphate form complexes with copper(II) and nickel(II). This suggests that the presence of a potentially coordinating amine or charged group (not attached to C-1) is a prerequisite. At high pH, monosaccharides with a blocked C-1 hydroxyl group, such as glucosamine pentaacetate and glucose 1-phosphate, do not form complexes. Also, because tetramethylglucose does not coordinate to Cu^{2+} at high pH, deprotonation of the C-1 hydroxyl group alone is not sufficient. The presence of stabilizing

groups (at the 2,3,4 or 6-positions) would appear necessary. In such cases, OH^- enters the first coordination sphere. For glucuronate and glucosamine, OH^- coordination does not occur due to the presence of the coordinating amine and carboxylate groups. The deprotonated glucosamine ligand thus resembles the glycinate ion in its coordinating behaviour.

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