The influence of pH on the coordination of copper(II) by 2-amino-2-deoxy-D-gluconate and 2-amino-2-deoxy-D-glucose oxime as studied by ^{1}H and ^{13}C NMR relaxation rate measurements and EPR spectroscopy

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Abstract

Copper(II) binding to the D-glucosamine derivatives 2-amino-2-deoxy-D-gluconate and 2-amino-2-deoxy-D-glucose oxime has been studied by ^{13}C and ^{1}H NMR relaxation rate measurements, visible and EPR spectroscopy, at two different pH values. From the Cu(II) induced relaxation rate enhancements distance information of the Cu(II) metal ion to the carbon nuclei of the ligands has been obtained, strongly affirming and further revealing the coordination mode of the ligands to Cu(II) as indicated by the visible and EPR measurements. It is shown that the coordination of the compounds is dependent on the pH. At pH 6.9, Cu(II) forms 1:2 complexes with 2-amino-2-deoxy-D-gluconate in which the copper is bound via two carboxylate and two amino groups at about equal distances, with the C3 hydroxyl groups probably hydrogen bonded to axially located water molecules. At pH 10.7 the ligand is interacting with the amino and C3 hydroxyl group in a nearly square plane and the carboxylate group is possibly in the axial position. Concerning the 2-amino-2-deoxy-D-glucose oxime only the *E* isomer coordinates to copper. At pH 6.9 Cu(II) complexes are formed wherein the ligand binds in a didentate fashion via the amino and oxime nitrogen. At pH 10.7, there is substantial contribution of a complex wherein the Cu(II) is monodentately bound via the oxime oxygen.

Introduction

The metal ion coordinating abilities of polyhydroxycarboxylates have been extensively studied by various techniques [1–8]. Metal ion sequestering properties of these compounds were found to increase upon addition of borate, the borate anion linking two ligands with the subsequent formation of strong metal ion coordinating sites [9–11]. Mixtures of borate and polyhydroxycarboxylates have been suggested as potential sodium triphosphate substitutes in detergent formulations [12, 13].

Recently we have studied the borate ester formation and metal ion sequestration of the D-glucosamine derivatives 2-amino-2-deoxy-D-gluconate (**1**) [14] and 2-amino-2-deoxy-D-glucose oxime (**2**) [15], see Fig. 1. Synergic sequestration of transition metal ions by the borate diesters of these ligands was elucidated using multinuclear NMR techniques and titration procedures, over the pH range 6–13. The results showed that Cd(II) binds to the borate diesters via two amino and two carboxylate groups (**1**) or via two amino and two oxime (or oximato) groups (**2**), irrespective of the pH. Cu(II), on the other hand, appeared to have a pH dependent coordination mode towards both **1** and **2**, for the effects of Cu(II) addition on the borate ester formation in mixtures of borate and **1** or **2** were pH dependent [14, 15].

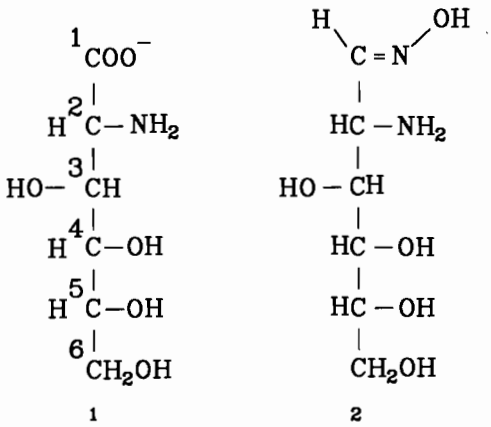

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Fig. 1. Structures of 2-amino-2-deoxy-D-gluconate (**1**) and the *E* isomer of 2-amino-2-deoxy-D-glucose oxime (**2**).

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Structural changes as a function of pH of coordination compounds of polyhydroxycarboxylates with metal ions have been discussed previously [16]. From the literature data, mostly obtained from potentiometric studies, a general coordination–ionization scheme, correlating the pH effects with the acidity of the hydrated cations, was proposed and the effects were explained using an extended electrostatic model [16]. The present study should be regarded as a continuation of our ongoing research on the coordination of metal ions to polyhydroxy compounds, whereby we have extended our work to ligands containing amino or oxime groups besides OH or carboxylate functionalities. EPR and visible spectroscopy measurements were used to determine the Cu(II) coordination modes of compounds **1** and **2**. Spin–lattice (T_1) and spin–spin (T_2) relaxation measurements in the presence of varying amounts of Cu(II), at different pH values, were used to further establish and obtain more direct information on a possible pH dependent Cu(II) coordination mode. ^{13}C and ^{1}H spin–lattice relaxation time (T_1) measurements are a powerful tool for studying metal ion–ligand interactions, as the paramagnetic Cu(II) ion induces relaxation rate enhancements of which distance information of the Cu(II) ion to the ligands can be obtained [17].

Experimental

Materials

2-Amino-2-deoxy-D-gluconic acid was prepared by the dehydrogenation of 2-amino-2-deoxy-D-glucose·hydrochloride on a 5% Pt/C catalyst in 0.33 M aqueous LiOH [18]. The compound was purified by recrystallization from methanol [14]. 2-Amino-2-deoxy-D-glucose oxime·hydrochloride was synthesized from 2-amino-2-deoxy-D-glucose·hydrochloride according to Finch and Merchant [19], and purified by recrystallization from methanol. 2-Amino-2-deoxy-D-glucose·hydrochloride was purchased from Janssen Chimica and $CuCl_2 \cdot 2H_2O$ was obtained from Aldrich. Cu(II) was added to solutions of the ligands in D_2O from stock solutions of 3.033×10^{-3} M (^{13}C NMR measurements) or 2.289×10^{-3} M (^{1}H NMR measurements) $CuCl_2 \cdot 2H_2O$ in deionized H_2O, using a microsyringe. The pH was adjusted with concentrated HCl or NaOH in D_2O. The pH values given are direct meter readings.

Visible spectroscopy measurements

Visible spectra were recorded, between pH 4 and 12, on a Pye Unicam SP-250 UV–Vis spectrophotometer at ambient temperature.

EPR measurements

X-band EPR spectra of frozen aqueous solutions of Cu(II) and **1**, **2** or L-serine at liquid nitrogen temperature (−196 °C), were measured with a JEOL JES-RE2X EPR spectrometer. Solutions were 3.1×10^{-3} M in Cu(II) and 3×10^{-2} M in **1**, **2** or L-serine. Frequencies were 9.104–9.106 GHz and microwave power was 4 mW. The g values were referenced to dpph ($g = 2.0023$).

NMR measurements

NMR spectra were recorded at 25 °C on a Varian VXR-400 S spectrometer at 100.6 (^{13}C) or 400.0 (^{1}H) MHz. Some spectra were also recorded on a Nicolet NT-200 WB spectrometer, at 50.3 MHz, for verifying that the fast exchange limit (eqn. (5), *vide infra*) was reached. ^{13}C spin–lattice relaxation times were measured by using a [180°-τ-90°-acq] inversion recovery pulse sequence, while for ^{1}H a [180-τ-12°-acq] inversion recovery pulse sequence was used. The T_1^{-1} values were calculated by using a three parameter fit of the experimental data [20]. The transverse relaxation rates were calculated from the linewidths at half-height by way of the relation $T_2^{-1} = \pi \Delta \nu_{1/2}$. These linewidths were determined by fitting the NMR signals with a Lorentzian line function. When necessary deconvolution was applied to obtain all the signal characteristics. The *E*:*Z* isomer ratios of the 2-amino-2-deoxy-D-glucose oxime were obtained from quantitative ^{13}C NMR spectra. The *E*:*Z* ratio appeared to be almost independent on the pH (3.8:1.0 at pH 6.9 and 4.0:1.0 at pH 10.7). Additional signals of a cyclic isomer ($\leqslant 8\%$), probably having a pyranose structure (similar to that observed for D-glucose oxime [19]) were detected in these spectra. The ρ values (ρ = Cu(II):ligand ratio) have been corrected for the presence of different amounts of *E* and *Z* isomers and the cyclic compound. Relaxation times of the cyclic isomer were only determined at pH 10.7 and they showed Cu(II) complexation to this compound to be negligible.

Theory

The paramagnetic contribution to the relaxation rate, T_{1p}^{-1}, in the presence of a small amount of paramagnetic metal ion, can be given by [21, 22]

$$T_{1p}^{-1} = T_{1(obs)}^{-1} - T_{1(0)}^{-1} = \rho q (\tau_m + T_{1,compl})^{-1} \qquad (1)$$

when the effects of the paramagnetic ion on the relaxation rate of nuclei beyond the first coordination sphere of the paramagnetic ion ($T_{1(os)}^{-1}$) are neglected. Here $T_{1(obs)}^{-1}$ is the observed spin–lattice relaxation rate, $T_{1(0)}^{-1}$ the relaxation rate in the absence of paramagnetic metal ions, ρ is the molar ratio of the paramagnetic ion to the ligand, q is the number of

ligands in the first coordination sphere, τ_m is the residence time of a nucleus within the first coordination sphere of the paramagnetic ion and $T_{1,\,compl}$ is the spin–lattice relaxation time of the nucleus in the complex. Equation (1) is known to reduce to $T_{1p}^{-1} = pqT_{1,\,compl}^{-1}$ for aqua complexes of paramagnetic ions of the first transition series as τ_m is small compared to $T_{1,\,compl}$. Then, according to the Solomon–Bloembergen equations [23, 24], the paramagnetic contribution to the spin–lattice relaxation rate for nuclei within the first coordination sphere in aqueous solutions is equivalent to

$$\frac{T_{1p}^{-1}}{pq} = T_{1,\,compl}^{-1} = \frac{2S(S+1)\gamma_I^2 g^2 \beta^2}{15r^6}\left(\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_s^2\tau_c^2}\right) + \frac{2S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left(\frac{\tau_e}{1+\omega_s^2\tau_e^2}\right) \quad (2)$$

S is the electron spin quantum number, γ_I is the nuclear magnetogyric ratio, g is the electronic 'g' factor, β the Bohr magneton, ω_I and ω_s the Larmor angular precession frequencies for the nuclear and electron spins, respectively, $A/\hbar$ the electron–nuclear hyperfine coupling constant, τ_c the dipolar correlation time, τ_e the scalar correlation time and r is the distance between the paramagnetic ion and the measured nucleus. The first term in eqn. (2) is due to the dipolar coupling between the unpaired electron residing at the metal ion and the resonating nuclei. The second, scalar, term is due to unpaired spin delocalization onto the ligand. A third contribution to the relaxation rate, especially present in π-bonding systems [17], and not included in eqn. (2), results from the dipolar coupling between unpaired spin density on non-s-orbitals of the ligand and the resonating nuclei. These ligand centered effects, however, are hard to evaluate [17, 25] but are more important for ^{13}C than ^1H nuclei. By making the assumptions that $\omega_I < \tau_c^{-1} < \omega_s > \tau_e^{-1}$, which is true for paramagnetic ion complexes of small ligands [17], eqn. (2) simplifies to

$$T_{1,\,compl}^{-1} = \frac{6S(S+1)\gamma_I^2 g^2 \beta^2 \tau_c}{15r^6} \quad (3)$$

The spin–spin relaxation rate T_2^{-1} can be given by formula (4) [17]

$$T_{2(obs)}^{-1} = pq(\tau_m \Delta\omega_m^2 + T_{2,\,compl}^{-1}) + T_{2(0)}^{-1} \quad (4)$$

where τ_m is the lifetime of a ligand bound to the metal ion and $\Delta\omega_m$ is the chemical shift difference between the bound and unbound ligand resonances. $T_{2,\,compl}^{-1}$ in eqn. (4) is equivalent to

$$T_{2,\,compl}^{-1} = \frac{7S(S+1)\gamma_I^2 g^2 \beta^2 \tau_c}{15r^6} + \frac{S(S+1)\tau_e}{3}\left(\frac{A}{\hbar}\right)^2 \quad (5)$$

For spin–spin relaxation rates the scalar term often predominates and no distance information can be obtained from T_2 measurements [17]. However, when the fast exchange limit holds, *i.e.* $T_{2,\,compl}^{-1} \gg \tau_m \Delta\omega_m^2$, eqn. (4) reduces to $T_{2(obs)}^{-1} = pqT_{2,\,compl}^{-1} + T_{2(0)}^{-1}$, and the values of the hyperfine electron–nuclear interaction constants can be determined by substitution in eqn. (5) of the distances calculated via eqn. (3). These constants may give some additional information on the complexation of Cu(II) to the ligands.

Results and discussion

Visible absorption measurements

Visible absorption spectra of solutions containing 6 mM copper(II) and 15 mM 2-amino-2-deoxy-D-gluconate (**1**) were recorded between pH 4 and 12. Broad absorptions were observed. Upon increasing the pH the λ_{max} gradually shifted from 628 (pH 4.7–5.5) to 610 (pH 10.7) nm, whereupon the solution turned from blue to violet. Solutions of 5 mM Cu(II) and 6.5 mM 2-amino-2-deoxy-D-glucose oxime (**2**) showed broad absorption bands with a λ_{max} of 595 nm at pH 7.8 and λ_{max} of 580 nm at pH 9.8–11.4. For **1** the λ_{max} values are typical of amino acid complexes of copper(II) with two nitrogen and two oxygens coordinated to the metal ion in a square planar geometry [26]. The λ_{max} values of **2** indicate coordination by predominantly nitrogen ligands [27]. The visible absorption spectra indicate the formation of Cu(II)–ligand 1:2 complexes, but it is not possible to discriminate between a *cis*- or *trans*-ligand geometry in these complexes on the basis of the spectra.

EPR measurements

X-band EPR spectra of frozen solutions (−196 °C) of mixtures of Cu(II) and a tenfold excess of 2-amino-2-deoxy-D-gluconate (**1**) or 2-amino-2-deoxy-D-glucose oxime (**2**) showed only a single absorption signal indicating that the copper(II) complexes formed were mononuclear. The values of $g_\perp$, $g_\parallel$ and $A_\parallel$ (Table 1; no $A_\perp$ hyperfine splittings were observed) depend on the strength and degree of covalency of the metal–ligand bond and hence on the nature of the donor atoms [26, 28]. For square planar Cu(II) complexes g values generally decrease along the sequence $O_4 \rightarrow O_2N_2 \rightarrow N_4$ donor atoms [29], whereas A values increase. The $g_\parallel$ and $A_\parallel$ value of **2** are very close to that observed for Cu(II)–diamine 1:2 complexes [30], showing that the Cu(II) atom is coordinated by four nitrogen atoms.

EPR spectra of **1** at both pH 7.2 and 11.3 were very similar to those recorded for L-serine and spectra reported for 1:2 complexes of Cu(II) and other amino acids [26, 30].

For **1** the EPR spectra showed differences between pH 7.2 and 11.3 (see Table 1) indicating a pH dependent variation of the coordination. The larger copper hyperfine constants ($A_{\parallel}$) and smaller $g_{\parallel}$ and $g_{\perp}$ values at high pH reflect a more covalent metal–ligand bond which, for α-hydroxy amino acids like serine and threonine, previously has been explained as arising from Cu(II) coordination in a tridentate fashion, by the interaction of the α-hydroxyl group (C3 of **1**) with the Cu(II) atom at high pH [26].

For **2**, EPR spectra at pH 7.2 and 10.9 showed only a small decrease of the $A_{\parallel}$ value at higher pH, which suggests that in that compound no interaction of the Cu(II) metal ion with the (C3–C6) hydroxyl groups occurs at high pH.

^{13}C and ^{1}H NMR relaxation measurements; 2-amino-2-deoxy-D-gluconate (1)

^{13}C and ^{1}H spin–lattice relaxation rates of 2-amino-2-deoxy-D-gluconate (**1**) were measured at two different pH values and at various molar ratios Cu(II) to ligand (ρ). A linear relationship of $T_{1(obs)}^{-1}$ with ρ was found for both the ^{13}C and ^{1}H measurements (correlation coefficient >0.99 for C1–C3, see Fig. 2 for pH 6.9). The large differences between the Cu(II) induced relaxation rate enhancements for the different nuclei show that the assumption that τ_m in eqn. (1) can be neglected is indeed valid. Hence, $T_{1,\,compl}^{-1}$ values for the nuclei (Table 2) can be obtained from the slopes of the lines.

The stability constants of **1** with Cu(II) have been reported to be log β_1 (ML/M·L) = 8.07 and log β_2 ($ML_2/M \cdot L^2$) = 14.76 (I = 0.05, 25 °C) [31]. Therefore, under the conditions applied ($\rho < 15 \times 10^{-4}$), at pH 6.9 Cu(II) is almost exclusively bound as a 1:2 complex. A 1:2 complex at pH 6.9 is in line with the sequestering values found at this pH [14]. The sequestering values at pH 10.7 and at about nearly equal molar ratios Cu(II):ligand, however, indicate a different stoichiometry of the Cu(II) complexes suggesting the existence of CuL and possibly Cu_2L complexes at high pH. Complexes of such stoichiometry have been suggested for D-gluconate [8]. Under the high ligand:Cu(II) ratios applied for measuring the ^{13}C relaxation rates, however, the predominant complex is most likely that with the highest coordination of the copper ion and therefore the contribution of CuL and Cu_2L complexes to the relaxation rate can be neglected. The EPR measurements which showed that the Cu(II) complexes are mononuclear further justify the latter assumption.

From the $T_{1,\,compl}^{-1}$ values (Table 2) distances of the copper ion to the proton and carbon nuclei can be calculated, with the use of eqn. (3), if the correlation times, τ_c, of the complexes are known. Another approach is the assumption of a Cu–N distance in the complexes of **1** with Cu(II) and subsequent calculation of τ_c and the other distances by employing eqn. (3). In bis(L-serinato)copper(II) crystals, the Cu–N distance is 1.988 Å [32], which prompted us to take a Cu–N distance of 2.00 Å for the Cu(II) complexes of **1**. This leads to a Cu–C2 distance of 2.95 Å and a Cu–H2 distance of 3.90 Å. Using these distances, from the $T_{1,\,compl}^{-1}$ values of Table 2 it can then be calculated from eqn. (3) that these data are consistent with a τ_c value, obtained from the ^{1}H data, of 2.2×10^{-10} s and a τ_c value, obtained from the ^{13}C data, of 1.9×10^{-10} s. These τ_c values agree very well and suggest that although ligand centered effects may be present, they do not play an essential role in the determination of the (relative) Cu(II)–proton or Cu(II)–carbon nuclei distances. Furthermore, as τ_c values for low molecular weight complexes of Mn(II) and Cu(II) are usually of the order 10^{-10} s [17], these values seem very reasonable and all distances for the Cu(II) complexes of **1** have been calculated using $\tau_c = 2.1 \times 10^{-10}$ s.

The calculated distances given in Table 3, show that besides H2 and C2 also for the other proton and carbon nuclei the ^{13}C and ^{1}H data are coherent and show that at pH 6.9 the Cu(II) is bound to **1** via two amino and two carboxylate groups at about equal distances, whereas the Cu–C3 distance is 1.2 times larger. Dreiding models show that these calculated distances are in agreement

TABLE 1. EPR parameters observed for aqueous solutions of 3.1 mM Cu(II) and 3×10^{-2} M **1**, **2** or L-serine at −196 °C

Ligand	pH	$g_{\perp}$	$g_{\parallel}$	$A_{\parallel}$ (gauss)[a]
L-Serine	7.0	2.076	2.250	165
	11.2	2.056	2.234	182
2-Amino-2-deoxy-D-gluconate (**1**)	7.2	2.076	2.250	170
	11.3	2.057	2.226	193
2-Amino-2-deoxy-D-glucose oxime (**2**)	7.2	2.061	2.195	203
	10.9	2.058	2.200	192

[a]No $A_{\perp}$ hyperfine splittings were observed.

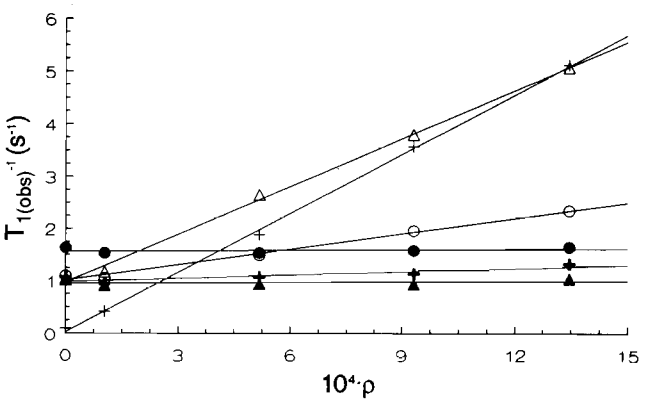

Fig. 2. Cu(II)-induced ^{13}C relaxation rate enhancement of 2-amino-2-deoxy-D-gluconate as a function of the Cu(II) to **1** ratio (ρ), at pH 6.9 and 25 °C. △ = C1, + = C2, ○ = C3, + = C4, ▲ = C5, ● = C6.

TABLE 2. $T_{1,compl}^{-1}$ values. 10^{-3} (in s^{-1}) for the ^1H and ^{13}C nuclei of 2-amino-2-deoxy-D-gluconate (**1**) and the ^{13}C nuclei of the *E* isomer of 2-amino-2-deoxy-D-glucose oxime (**2**) at pH 6.9 and 10.7, assuming $q = 2$

	1		**2**	
	pH 6.9	pH 10.7	pH 6.9	pH 10.7
C1	1.89	0.949	1.52	2.45
C2	1.53	1.53	0.751	0.521
C3	0.495	1.54	0.280	0.096
C4[a]	0.11	0.27		
C5[a]	0.017	0.17		
C6	0.02	0.06	0.02	0.01
H2	5.19	5.59		
H3	2.13	4.05		
H4	0.21	1.31		
H5	0.19	1.16		
H6, H6′ [b]		0.5		

[a]For **2** signals of C4 and C5 coincided. [b]$T_{1,compl}^{-1}$ values were similar for H6 and H6′.

with a complex structure in which the Cu(II) binds to **1**, in an octahedral 1:2 complex, via two amino and two carboxylate groups in an approximately square planar geometry, with the C3 hydroxyl groups probably hydrogen bonded to axially coordinated water molecules, (Fig. 3(a)). At pH 10.7 the calculated distances between Cu(II) and the carbon nuclei differ from that at pH 6.9. Now the results are consistent with a 1:2 complex with the Cu(II) binding via two amino and two deprotonated C3 hydroxyl groups in a square plane. The carboxylate groups might occupy the weaker coordination sites at the axial positions (see Fig. 3(b)). The gluco configuration at C2 imposes *cis*-ligand geometry in these complexes (see Fig. 3).

Once the distances of the Cu(II) ion to the various ligand nuclei have been determined, the values of the electron–nuclear hyperfine interaction constants can be

TABLE 3. Distances between the proton and carbon nuclei of 2-amino-2-deoxy-D-gluconate (**1**) or the carbon nuclei of the *E* isomer of 2-amino-2-deoxy-D-glucose oxime (**2**) and Cu(II) in Å ($q = 2$)[a,b]

	1		**2**	
	pH 6.9	pH 10.7	pH 6.9	pH 10.7
C1	2.85	3.19	2.62	2.42
C2	2.95	2.95	2.95	3.14
C3	3.56	2.95	3.47	4.16
C4[c]	4.6	3.9		
C5[c]	6.3	4.3		
C6	6.1	5.0	5.7	4.1
H2[d]	3.90	3.84		
H3	4.5	4.1		
H4	6.6	4.9		
H5	6.7	5.0		
H6, H6′	>7	5.7		

[a]Assignments of the ^{13}C NMR signals for **1** and **2** were made by recording ^{13}C NMR spectra as a function of pH and using established chemical shift vs. pH relations [33, 34]. [b]Distances were calculated using $6S(S+1)\gamma_I^2 g^2 \beta^2/15 = 8.28 \times 10^{-44}$ m^6 s^{-2} (eqn. (3)) for protons and 5.23×10^{-45} m^6 s^{-2} for carbon nuclei. [c]For **2** signals of C4 and C5 coincided. [d]^1H NMR signals were assigned by analyzing the vicinal and geminal ^1H coupling constants.

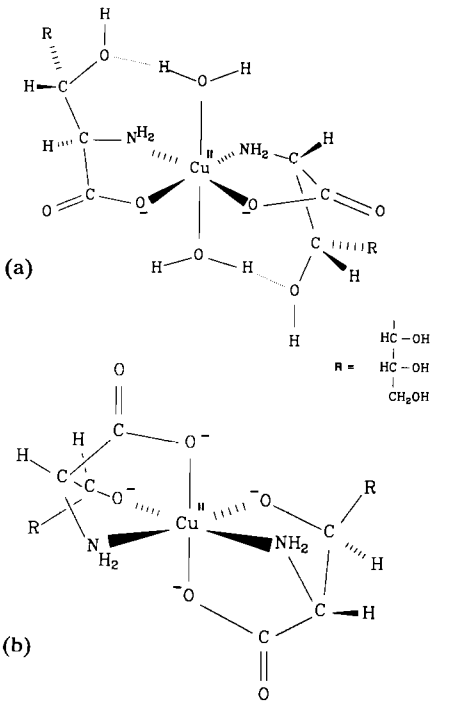

Fig. 3. Structures for the Cu(II) complexes of **1** at pH 6.9 (a) and 10.7 (b).

calculated if eqn. (5) can be employed. However, at pH 6.9, linewidths for the different carbon nuclei of **1** were substantially larger at 100.6 MHz than at 50.3 MHz. The increase in linewidths at higher frequency

indicates that the term $\tau_m \Delta\omega_m^2$ in eqn. (4) cannot be neglected and hence no electron–nuclear hyperfine interaction constants can be calculated from eqn. (5). This behavior is in contrast to ^{1}H NMR relaxation rate studies of Cu(II)–amino acid complexes [17, 35], where linewidths were found to be identical at different fields. For **1** the linewidths at both fields were much larger at higher pH. A similar pH dependent line broadening in ^{1}H NMR spectra of Cu(II)–glycine solutions was observed by Beattie *et al.* [35].

2-Amino-2-deoxy-D-glucose oxime (2)

^{13}C spin–lattice relaxation rates of **2** were measured at both pH 6.9 and 10.7, at various Cu(II) to ligand ratios. Under the conditions used signals for C4 and C5 were found to coincide. Signals for C6 of the *E* and *Z* isomer coincided only at pH 6.9. As for **1**, a Cu–N distance of 2.00 Å in the Cu(II) complexes of *E*-**2**, at pH 6.9, was assumed, a distance close to that found in crystals of Cu(II) complexes of α-amino oximes [36, 37]. From the induced paramagnetic relaxation rate for C2 of the *E* isomer (see Table 2) then a value for τ_c of 1.1×10^{-10} s was determined, which was used for calculating the distances given in Table 3. Though no stability constants have been reported for Cu(II) complexes of **2**, it is very likely that as for **1**, at the high ligand:Cu(II) ratios applied, a 1:2 complex is predominant. The resulting Cu(II) distances to the *E* isomer (Table 3) are consistent with didentate coordination of the Cu(II) via the amine and oxime nitrogen, whereas the 1:2 complex is probably stabilized by oxime–oximato hydrogen bonds [36, 37]. The Cu–C3 distance (as for **1** at pH 6.9) suggests that the C3 hydroxyl group probably has a hydrogen bond to axially coordinated water molecules (Fig. 4).

For the *Z* isomer, at pH 6.9, T_1 values were, within experimental error, independent of the amount of Cu(II) added. This reflects that monodentate coordination via the oxime or amino group (didentate coordination via the oxime and amino group is impossible for geometrical reasons) is absent or very weak. Moreover, as the relaxation rates for the carbon nuclei of the *Z* isomer remain constant upon adding Cu(II) at pH 6.9, the transfer of magnetization between the *E* and *Z* isomers apparently is negligible. The proposed didentate coordination of Cu(II) to the *E* isomer of **2** is in agreement with structures reported for α-amino oximes based on other techniques [36–39].

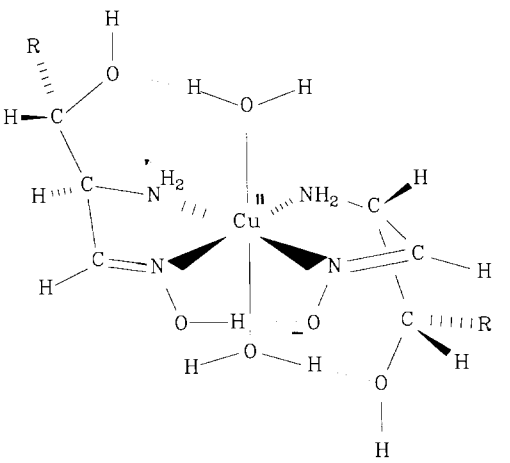

Fig. 4. Structure for the Cu(II) complex of the *E* isomer of **2** at pH 6.9

At pH 10.7, for the *E* isomer, the coordination mode of **2** to the Cu(II) ion is quite different from that for **1**. In contrast to **1**, the $T_{1,\text{compl}}^{-1}$ value of C1 increased and that of C3 decreased upon raising the pH, indicating that the Cu(II) now is interacting more with the oxime group than with the amino group and that didentate coordination with the oxime nitrogen and the amine as the predominant coordination mode is no longer compatible with the relaxation rates (Table 2). Although in the vast majority of oxime complexes coordination occurs at nitrogen, complexes where the metal ion is bound via the oximate oxygen are also known [40–43]. The EPR results (Table 1) indicate that at a high ligand to Cu(II) ratio the complexes are mononuclear in Cu(II). The increase of the calculated Cu(II) distances to C2 and C3 then can be best understood as resulting from an increased contribution of a complex in which Cu(II) is monodentately bound by the oximato oxygen. The interaction with the oximato oxygen at pH 10.7 can be explained by a loss of stabilizing oxime–oximato hydrogen bonds above pH 10, as a result of the ionization of the oxime hydroxyls of the free ligands (**2**: pK_a = NOH; 10.8 ± 0.2, D_2O, 25 °C [15]). Like for **1** linewidths at 100.6 MHz were significantly larger than at 50.3 MHz, indicating that eqn. (5) cannot be used and thus no electron–nuclear hyperfine interaction constants can be calculated.

For the *Z* isomer of **2**, at pH 10.7, the T_1 values of the carbon nuclei slightly decreased upon adding Cu(II). This increase of the relaxation rates of the carbon nuclei is unlikely to result from transfer of magnetization between the *E* and *Z* isomer as the relative magnitudes of the relaxation rate enhancements for the various carbon nuclei of *Z* isomer differ from that for the equivalent carbon nuclei of the *E* isomer. Hence, the results indicate a weak Cu(II) complexation to the *Z* isomer. The relaxation rate of C1 is affected most upon adding Cu(II), which indicates only monodentate complexation of this isomer via the oximato group.

Conclusions

For both 2-amino-2-deoxy-D-gluconate (**1**) and the *E* isomer of 2-amino-2-deoxy-D-glucose oxime (**2**) the

relaxation rate measurements confirm the indications obtained by visible and EPR spectroscopy and show that Cu(II) coordinates to the ligands in a fashion that depends on the pH. The involvement of the C3 hydroxyl in the Cu(II) complexation of **1** at higher pH, displacing the carboxylate functions to the weaker axial binding sites, will occur upon ionization of the hydroxyl function and reflects that an ionized alcoholic group is a stronger coordinating group than a carboxylate function. This pH dependent behavior is in line with the coordination–ionization scheme suggested for polyhydroxycarboxylates [16] which predicts a change from carboxylate to hydroxylate coordinating sites upon increasing the pH.

In contrast to **1**, the Cu(II) complexes of *E*-**2** show no tridentate coordination involving the C3 hydroxyl at higher pH. Apparently the oximato group is, at pH 10.7, a stronger coordinating group towards copper than the C3 hydroxyl group.

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