MIXED COMPLEXES OF COBALT(III) WITH D-GLUCOSAMINE AND PHENANTHROLINE

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ABSTRACT

Examination of ^1H chemical shifts of a complex of D-glucosamine with bis-phenanthroline cobalt (III) shows that the dominant Δ-complex is formed from α-D-glucosamine. A molecular mechanics simulation of the structure of this complex indicates that the sugar residue is not markedly perturbed by complexation of Co(III) at the 1-alkoxide and 2-amino groups. The circular dichroism spectrum of the complex formed in solution agrees with that of the isolated material.

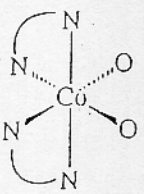
RESUMEN

El examen de los desplazamientos químicos ^1H de un complejo formado por D-glucosamina y Co(III) bis-fenantrolina muestra que el complejo predominante corresponde a la configuración Δ con el anómero α de la D-glucosamina. La simulación de estructura de este complejo por cálculos de mecánica molecular indica que el azúcar no se perturba mayormente al complejar al Co(III) a través de los grupos 1-alcóxido y 2-amino de este hidrato de carbono. El espectro de dicroismo circular del complejo formado en solución concuerda con el del compuesto aislado.

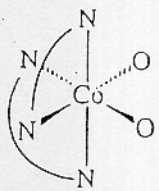
INTRODUCTION

D-Glucosamine (2-amino-2-deoxy-D-glucopyranose) forms complexes with heavy metal ions[1,2] and mixed complexes of cobalt(III) with glucosamine and ammonia or diammines have been isolated[2,3]. Attention was focused on Co(III) complexes since its diamagnetism allowed us to use NMR spectrometry to examine structures of the sugar ligands[3]. In addition bis-diamine complexes with Co(III) are readily prepared and are stable over a range of conditions. The mixed complexes with 1,2-ethylene diamine (en) or 1,10-phenanthroline (phen) have the composition Co(III) : diamine : glucosamide = 1 : 2 : 1, so glucosamine is a bidentate ligand bonded through the amino and anomeric alkoxide residues[2]. Complexes of Co(III), D-glucosamine and en or phen are mixtures of diastereomers which can be separated chromatographically[2]. The configuration at Co(III) (scheme 1) can be determined from circular dichroism (CD) or optical rotatory dispersion (ORD) spectra[4,5]. For $[Co(III)(en)_2$D-glucosamine$]^{2+}$, and for $[Co(III)(phen)_2$D-glucosamine$]^{2+}$, Δ/Λ = 7:3 for a sample examined immediately after preparation but, Δ/Λ changes to 16:1[2] with time.

Scheme 1



Λ



Δ

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Complexation of sugars or cyclic 1,2 diols as bidentate ligands typically involves cis-ax, eq, groups[6,7] and on this basis Co(III) should be complexed to the eq-2-NH_2 and ax-α-1-OH/O^- residues. The ^{1}H-NMR spectrum of isolated Λ- [Co(III)$(en)_2$D-glucosamine]$^{2+}$ is consistent with complexation by the α-anomer[3]. The problem is that solid samples of these Co(III) complexes may slightly decompose generating small amounts of Co(II). These minor impurities do not significantly perturb elemental analyses or absorbance or CD spectra, but the paramagnetic Co(II) species produces considerable broadening of the NMR signals. The Co(III) $(phen)_2$ complexes with D-fructose[8] and L-sorbose[9] are unexpectedly more stable in solution than in the solid and good ^{1}H-NMR spectra can be obtained without isolation.

Rather poor quality ^{1}H-NMR spectra of isolated complexes of Co(III) $(phen)_2$ with D-glucosamine[2,3] were obtained so the measurements were carried out on complexes without isolation. This method involves a careful choice of conditions since if the solution is left for a long time, decomposition to Co(II) is observed. Since time is needed for complex formation, one has to make a compromise between conflicting requirements. Fortunately signals of H-1 and H-2 of the Co(III) complex are well separated from those of the sugar[3] and they allow unambiguous differentiation between the α- and β-diastereomeric complexes based on the magnitude of the H-1,2 coupling constant[10,11]. These coupling constants should be low for the α-anomer with cis-ax,eq,H-1,H-2 and high for the β-anomer with trans-ax,ax,H-1,H-2.

We used molecular mechanics calculations based on MM2 parameters to predict the enthalpies and preferred conformations of the complexes[3] and compared these predictions with the results from ^{1}H-NMR and CD spectrometry.

RESULTS AND DISCUSSION

NMR spectrometry.

The starting materials were cis[Co$(phen)_2Cl_2$]Cl [12] and D-glucosamine hydrochloride. In mildly alkaline solution the Co(III) chloride complex is readily hydrolyzed and then complexes with D-glucosamine[2] are formed.

$$[(phen)_2Co(III)Cl_2]^+ \xrightarrow{H_2O} [(phen)_2Co(III)(H_2O)_2]^{3+}$$

$$[(phen)_2Co(III)(H_2O)(OH)]^{2+} \xrightarrow{\text{D-glucosamine}} [(phen)_2Co(III)\text{D-glucosamine}]^{2+}$$

Four chloride ions are released in these reactions and high electrolyte broadens NMR signals. We therefore used dilute (0.01 M) reactants and unbuffered solutions to minimize electrolyte effects and possible complexation of Co(III) with buffers. Reaction does not go to completion under these conditions, but we obtained good ^{1}H-NMR signals of H-1 and H-2. We did not attempt to separate the other signals of the complex from those of the sugar. These conditions differ from those used in preparing complexes for isolation, where samples were left for several days at pH = 8 [2]. We obtained better NMR spectra but using a higher pH (pD) and much shorter times than those used for preparative work[2]. All experiments were made in D_2O at 25°C and chemical shifts, δ, are referred to TSP (sodium trimethylsilylpropionate d4). Data from reaction of 0.01 M reagents, pD = 9.8, 18 hr., are in Table I.

TABLE I. Chemical shifts of D-glucosamine and complexes with Co(III) $(phen)_2$ [a].

	H-1		H-2	
	δ,ppm	J, Hz	δ,ppm	J, Hz
α-Glucosamine	5.20d	3.5	2.71dd	3.5, 10.5
β-Glucosamine	4.57d	8.0	2.59ψt	8.5, 9.0
Major complex	5.38d	3.5	2.82dd	3.5, 11.5
Minor complex	5.58d	4.0	2.91dd	4.0, 10.0

[a]Chemical shifts relative to TSP in D_2O; pD = 9.8; 0.01 M reagents, after 18 hrs. at 25°C.

The chemical shifts and coupling constants show that the solution contained unreacted α and β sugars[10,11], and, based on the coupling constants, major and minor α-complexes. In this experiment, α-sugar:α-complex ≈ 2:1 and the minor complex was ca. 10% of the major. The extent of conversion into the Δ-complex is similar to that based on the CD spectrum in the visible region. We saw some very

minor signals, not due to the sugar (< 1% of the main signals of the Δ-complex). In another experiment with 0.01 M reagents, pD = 9.0, signals of the major α-complex after 4 hrs. were observed, but so little complex was formed that we did not see a minor complex. Under these conditions signals of the complex were at 5.40 ppm, d(H-1) and 2.83 ppm, d,d (H-2), and the dominant signals were those of the sugar. In both sets of experiments signals began to broaden significantly within 2 days.

Assignments of these signals are straightforward[3,10,11] although we note that the chemical shifts of glucosamine are pH dependent[3]. In fact, at low pH, protonation increases the chemical shifts and the anomeric OH group should be deprotonated at high pH, in similar fashion as other sugars[13]. In addition chemical shifts are affected by electrolyte. Our present values of δ differ from those quoted earlier which were referred to δ_{HDO} = 4.63 ppm, and were measured in excess KOD, so the extent of deprotonation is uncertain[3]. Values of δ_{HDO} = 4.81, referred to DSS and TSP respectively, are now preferred to the earlier value (Cambridge Isotope Lab., Andover, Mass).

The α sugars and their complexes have H-1 and H-2, cis, eq-ax, which gives relatively low coupling constants, whereas in the β-sugars H-1 and H-2 are trans, ax-ax, with high coupling constants[10,11]. The signal of H-2 of α-glucosamine is a doublet of doublets whereas that of β-glucosamine is a degenerate doublet of doublets and appears as a pseudo-triplet because of peak overlap[3].

Circular dichroism spectrometry

We examined the CD spectrum of a 1:1 mixture of 0.01 M D-glucosamine and $[Co(III)(phen)_2Cl_2]^+$ at pH 9.2 under conditions similar to those used for the NMR experiments, i.e., 18 hrs at 25°C in order to confirm formation of the Δ-complex in the NMR experiments. The sign sequence and position of the peaks are very similar to those found for a sample of the Δ-complex isolated chromatographically[2]. Amplitudes of the CD signals increase with time[2] and the UV and visible CD spectra were taken after 3 days at 25°C.

The CD signal in the visible region at 497 nm (negative) is located under the first d→d absorption band of Co(III), corresponding to the dominant ^1E state[2]. The positive signal at 413 nm is under the second d→d absorption band and corresponds to the $^1T_{2g}$ state which is masked by the intense absorptions of phenanthroline.

There are strong absorbances of the phenanthroline ligands in the UV region, but due to problems of signal to noise, extrema in the CD spectrum were not observed earlier at λ < 236 nm[2]. We have now observed additional signals. Signals in the UV region are at ca. 320 nm (positive), 280 nm (negative), 262 nm (positive). The last two signals correspond to the excitonic effect which gives evidence on the configuration of the predominant isomer. The other UV signals are at 245 nm (weak negative), 234 nm (positive), 225 nm (negative) and 214 nm (negative).

These CD spectra are of a mixture of Δ and Λ complexes, but after equilibration Δ/Λ ≈ 16, so these spectra are a reasonable approximation of those of Δ-Co(III) $(phen)_2$-α-D-glucosamine[2].

Effect of phenanthroline on chemical shifts

Complexation with Co(III) $(phen)_2$ affects chemical shifts of glucosamine in different ways. Coordination of the amino groups and of oxygen at position 1 increases chemical shifts of H-2 and H-1 by an inductive effect. However, diamagnetic shielding strongly reduces chemical shifts of hydrogens close to the face of a phenanthroline ring[8,9,14,17] and this shielding is very marked in complexes of D-fructose[9] or L-sorbose[9] with Co(III)$(phen)_2$. Hydrogens adjacent to the edges of the rings show increased chemical shifts. Inspection of Fig. 1 shows that in the Δ-complex with α-glucosamine neither H-1 nor H-2 is close to a ring face, consistent with the observed differences in the chemical shifts of α-glucosamine and the major Δ-complex given in Table I. Phenanthroline is a very useful ligand in the study of mixed complexes because it is conformationally rigid and its ^{1}H-NMR signals are well to the left of signals of aliphatic hydrogens and do not interfere with them. Observation of diamagnetic shielding can provide useful information on conformations of ligands[8,9,14,17].

As noted earlier we used different conditions for the preparation and isolation of complexes of D-glucosamine and Co(III) $(phen)_2$ and for the NMR work[2]. In the preparative work at pH 8 initially Δ:Λ = 7 : 3, but with time, and after chromatography, Δ:Λ = 16 : 1 [2]. Probably the initial mixture was kinetically and the latter was thermodynamically controlled. Our NMR experiments were in D_2O, rather

than H_2O, and the pD was significantly higher than the pH of the earlier preparative experiments[2]. This higher pD should increase equilibration of interconverting diastereomers, resulting in our seeing one dominant complex.

Molecular modelling

The configuration of Co(III) of the dominant complex is established as Δ by CD spectrometry[2] and the simulated structure is shown in Fig. 1. This complex is predicted to have a lower enthalpy (by ca. 1 kcal•mol^{-1}) than Δ-Co(III)(phen)$_2$-β-glucosamine, and the Λ complexes. However, the simulation neglects hydration (experimental) so the predicted enthalpies are not reliable.

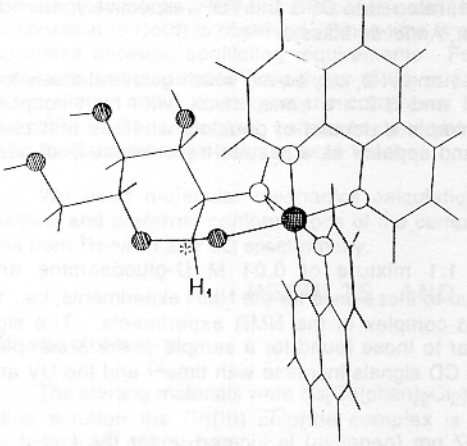

FIG. 1. Simulated structure of the complex of Co(III)(phen)$_2$ and α-D-glucosamine, Co, ●; O, ◎; N, O ; ✱ , anomeric center. Carbon atoms are not shown and carbon-hydrogen bonds are indicated by sticks.

The predicted dihedral angles of the complex, H-1,2, 41° and H-2,3, 166° are consistent with the observed coupling constants (Table I) based on the Karplus relation[14]. The other bond and dihedral angles (Experimental) indicate that the sugar ring is not significantly distorted by complexation, although we could not test this point by determining the coupling constants at other positions in the complex because of extensive overlap with signals of the sugar and low conversion of glucosamine into complex.

In the simulation coordination is shown as involving alkoxide oxygen at position 1, because coordination of hydroxyl groups to heavy metal ions strongly increases acidities[15,16].

EXPERIMENTAL

Materials, preparation and NMR spectra

The bis-phenanthroline complex was prepared in D_2O from trans-[Co(phen)$_2$Cl$_2$]Cl [12]. This complex aquates and isomerizes to the cis-complex which reacts relatively slowly with D-glucosamine[2]. The pD of the solution was adjusted to 9.4 with KOD. The optimum conditions for examining the ^{1}H-NMR spectrum of the complex involved low reagent concentrations (0,01 M) in order to limit the size of the HDO signal and electrolyte effects, and shorter reaction times than those used preparatively[2]. For the spectrum run after 4 hrs, solvent suppression was used but results were similar to those obtained in other experiments without solvent suppression. Formation of paramagnetic Co(II) leads to significant deterioration of the NMR spectra due to line broadening. It occurs if the Co(III) complexes decompose by disproportionation or aquation. Under these conditions new signals appear and all signals are very broad. We made one tentative experiment with glucosamine and Co(III) (en)$_2$ following the general method described above but only broad signals were detected.

NMR spectra

(500 MHz) were measured at 25°C in a GN 500 spectrometer. Mutarotation of D-glucosamine is relatively rapid in the conditions of our experiments and for the uncomplexed sugar $\alpha/\beta \approx 0.4$, in reasonable agreement with other NMR evidence[3] and results in H_2O [18].

There is considerable overlap between signals of H-3, H-4, H-5, H-6 and H-6' of α- and β-glucosamine and the Λ-complex with Co(III) $(en)_2$ [3]. We did not attempt to assign these signals of the complex with Co(III) $(phen)_2$, because the signals of H-1 and H-2 of the complex provide complete information on the configuration at position 1 of the sugar.

Based on the ratios of α-sugar/α-complex = 2 and, for the sugars, $\alpha/\beta \approx 0.4$ [3,18] we obtain ca. 15% overall conversion of D-glucosamine into the Δ-complex, in reasonable agreement with CD data.

CD spectra

Solutions were made up in H_2O, pH 9, but otherwise conditions were those used in the NMR experiments. The spectra were monitored in a Jobin-Yvon DC6 spectrometer. Concentrations were similar to those used for the NMR work.

After 18 hrs at 25°C with 0.01 M reagents at pH 9.0 ΔAbs for the negative CD signal at 497 nm is ca. -7.5×10^{-3} in a 1 cm cell and for isolated Δ-complex[2] $\Delta\varepsilon = -3.4$ corresponding to approximately 20% conversion of sugar into complex. This estimation neglects the presence of some Λ-complex[2], but is in reasonable agreement with the extent of conversion based on NMR spectrometry, in view of possible isotope effects and differences in the pH and pD scales. If we base our estimation on the positive CD signal at 238 nm with $\Delta Abs \approx 0.8 \times 10^{-3}$ and $\Delta\varepsilon = 49.4$ [2] we calculate 16% conversion of sugar into complex.

Structure simulations

The MM2 program was used on a Cache Tektronix computer (Tektronix, August 1991, Version 2.7)[3,8]. The MM2 parameters are: relaxation factor = 1; convergence to 10^{-3} kcal•mol^{-1} and energy terms include: bond angles and stretch, dihedral angles and improper torsions, intramolecular van der Waals, electrostatic and hydrogen-bonding interactions. Medium effects and hydrogen-bonding with water are neglected but their contributions should be similar for the various diastereomers. The only geometrical restriction was that glucosamine had the pyranose structure.

In the predicted structure of Δ-Co$(phen)_2$ α-glucosamine bond lengths to Co(III) were: Co-O, 2.15; Co-NH_2, 2.16 and Co-N(phen), 1.92 Å in reasonable agreement with crystallographic data in other Co(III)complexes[19,20]. The bond angle at Co(III) to the amino and alkoxide moieties was 83° and H-H dihedral angles of the sugar were: H-1,2, 41°; H-2,3, 166°; H-3,4, 170°; H-4,5, 179°; H-5,6, 51° and H-5,6', 175°. These values are similar to those predicted for α-glucosamine[3] and fit its ^1H coupling constants so the bonding to Co(III) does not significantly perturb the conformation of the pyranose ring. We neglect solvation in this calculation, but Bagley *et al.*[21] have made a molecular dynamics simulation of sucrose including solvent, either H_2O or DMSO, and concluded that structures in solution are similar to that in the crystal.

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