



PS5.40 — TU

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## SPECTROSCOPIC AND POTENTIOMETRIC STUDIES OF COPPER(II) COMPLEXES OF D-GLUCOSAMINE

Because of the involvement in many bioinorganic systems, the interaction of metal ions with simple amino sugars such as D-glucosamine has been studied [1,2], but no precise description of the complexes is really available. We have investigated the Cu(II)-D-glucosamine system in aqueous solution by using spectroscopic (ESR, absorption and CD) and potentiometric techniques.

According to the potentiometric results, five distinct complex species are formed over the pH range 5-9.5 (Table I).

The  $\text{CuL}_2$  complex, the major species around pH 7 (~55% at pH 6.9), is easily distinguished by spectral measurements. Indeed, the *d-d* absorption maximum (660 nm,  $\epsilon=44$ ), the ESR parameters ( $g_{\parallel}=2.317$ ,  $A_{\parallel}=175 \times 10^{-4} \text{ cm}^{-1}$ ) and the CD data (640 nm,  $\Delta\epsilon=+0.06$ ) strongly support the involvement of two nitrogen atoms in metal coordination [3-4].

Above pH 7 two other complexes, namely

Table I

Logarithm of stability constants ( $\log \beta_{pqr}$ ) of complex species  $M_p H_q L_r$  ( $M=\text{Cu(II)}$ ,  $L=\text{D-glucosamine}$ ) in 0.15 M NaCl at 25°C

p	q	r	$\log \beta_{pqr}$
0	1	1	7.70
1	0	1	3.06
1	0	2 ( $\text{CuL}_2$ )	8.76
1	-1	2	0.83
1	-2	2 ( $\text{CuH}_{-2}\text{L}_2$ )	-5.82
1	-3	2	-15.08

$\text{CuH}_{-1}\text{L}_2$  (~10% at pH 7.4) and  $\text{CuH}_{-2}\text{L}_2$  (~90% at pH 8.1), are formed. The minor species,  $\text{CuH}_{-1}\text{L}_2$ , which is not shown by any used spectroscopic technique, results from the deprotonation of one of the hydroxyl groups of a glucosamine ligand and, thereby, involves a chelate (N,O) ring. The  $\text{CuH}_{-2}\text{L}_2$  species ( $\lambda_{\text{max}}=620 \text{ nm}$ ,  $g_{\parallel}=2.255$ ,  $A_{\parallel}=196 \times 10^{-4} \text{ cm}^{-1}$ ) involves two chelate (N,O) rings because of the coordination of two amino groups and two deprotonated hydroxyls. The formation of  $\text{CuH}_{-2}\text{L}_2$  gives rise to strong negative CD effects centered around 730 nm ( $\Delta\epsilon=-0.15$ ).

Positive Cotton effects, attributable to  $\text{NH}_2 \rightarrow \text{Cu(II)}$  charge transfer transitions, are observed in the UV region ( $\text{CuL}_2$ : 315 nm,  $\Delta\epsilon=+0.6$ ;  $\text{CuH}_{-2}\text{L}_2$  at pH ~8: 300 nm,  $\Delta\epsilon=+2.2$ ).

The formation of  $\text{CuH}_{-3}\text{L}_2$ , which predominates above pH 10, does not result in any distinguishable variation of absorption or ESR spectra.

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PS5.41 — TH

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### ENDOR STUDY OF SMALL MOLECULE AND ENZYME COMPLEXES OF $Gd^{3+}$ IN FROZEN SOLUTION

Electron-nuclear double resonance (ENDOR) spectroscopy is applied to the investigation of the coordination structure of  $Gd^{3+}$  in small molecule and enzyme complexes in frozen solution. Proton ENDOR spectra of  $GdCl_3$  in frozen methanol-water mixtures obtained with  $H_0$  at the turning point of the EPR absorption exhibit single crystal-type line pairs. With use of selectively deuterated solvents, we have assigned the chemical origins of each pair of ENDOR lines. There are two distinguishable sets of protons due to metal-coordinated water and one set belonging to the methyl group of metal-coordinated methanol. Similarly, from the proton ENDOR spectrum of  $Gd(CH_3COO)_3$  in frozen solution, we have also identified the set of lines belonging to the methyl group of metal-bound acetate. On the basis of the field dependence of the ENDOR spectra, we have determined the hyperfine coupling (hfc) components of each of the metal-bound ligands.

The hfc components of the protons of  $Gd^{3+}$ -bound acetate exhibit axial symmetry, and under the point-dipole approximation, the calculated metal-proton distance is  $4.53 \pm 0.20$  Å. This is in reasonably good agreement with the value of 4.73 Å deduced from crystallographic data. The hfc components of the water and methanol protons do not exhibit axial symmetry, indicating significant spin delocalization. Nonetheless, the metal-proton distances, calculated as lower limit estimates on the basis of the largest anisotropic

hfc component, are in surprisingly reasonable agreement with crystallographic data. Application of this method is made to investigate the environment of lanthanide binding sites in enzymes and proteins.



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### A NMR STUDY OF $Ln(NOTA)$ CHELATES AS AXIALLY SYMMETRIC AQUEOUS SHIFT REAGENTS

The trivalent lanthanide ethylenediaminetetraacetate ( $Ln(EDTA)$ ) chelates are very useful water soluble NMR shift and relaxation probes of the dynamic structure of biological molecules at pH ~ 7 [1], including nucleotides [2,3], carboxylates [4,5] and aminoacids [6]. The  $Ln(EDTA)$  complexes suffer several disadvantages as aqueous NMR shift reagents including lack of solubility near neutral pH, exchange broadening of the resonances of some ligands to the heavier  $Ln(EDTA)$  chelates [6] and structural changes along the series, which complicates the separation of pseudocontact and contact shifts [7,8].

In this work we report  $^{13}C$  and  $^1H$  NMR studies of the 1:1 complexes of the axially symmetric macrocyclic ligand 1,4,7-triazacyclononane- $N,N',N''$ -triacetic acid (NOTA) with the diamagnetic and