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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 ± 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 $pH \sim 7$ [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

paramagnetic lanthanides. The proton and ^{13}C spectra of the $\text{La}(\text{NOTA})$ and $\text{Lu}(\text{NOTA})$ species were studied as a function of pH and temperature. The aqueous complexes show spectra characteristic of a flexible triaza cycle, displaying fast interconversion between the two staggered δ and λ conformations of the ethylenediamine rings even at room temperature. Above pH 9.5, the hydroxo complexes are formed, and their spectra show evidence for a much more rigid triaza macrocycle. At room temperature the conformational interconversions are slow in the NMR time scale. Proton and ^{13}C NMR dynamic studies gave a value of $\Delta G^\ddagger = 63.6 \text{ KJ mol}^{-1}$ for the activation energy of this process.

LIS values have been measured for all proton and carbon resonances in nine paramagnetic $\text{Ln}(\text{NOTA})$ complexes (Fig. 1). The ethylene protons appear as a pair of resonances forming an

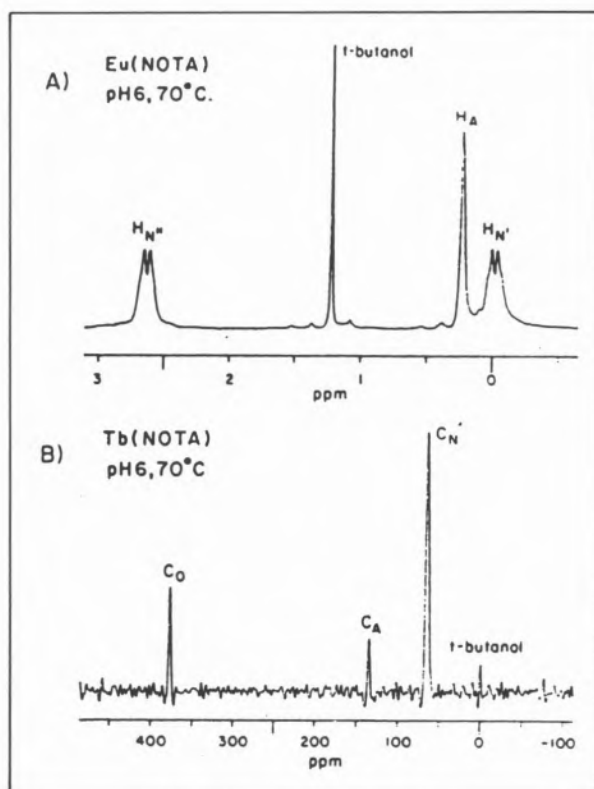


Fig. 1

Proton(A) and carbon(B) NMR spectra of $\text{Ln}(\text{NOTA})$ complexes

$\text{AA}'\text{XX}'$ splitting pattern (visible only in the $\text{Eu}(\text{NOTA})$ spectrum) while the acetate protons remain a singlet. The observed LIS's are domi-

nated by contact interactions in most of the $\text{Ln}(\text{NOTA})$ complexes.

The ^{13}C spectrum of $\text{Pr}(\text{NOTA})$, studies of the effect of added LiCl on the observed LIS's and observed breaks in plots of experimental LIS data versus theoretical pseudocontact and contact shift values, suggest that the early members of the lanthanide ion series form mixed complexes with NOTA in aqueous solution, some with NOTA bound as a hexadentate chelate and some as a pentadentate species with one unbound acetate group. The smaller trivalent lanthanide cations ($\text{Dy} \rightarrow \text{Yb}$) appear to form complexes containing only hexadentate chelated NOTA .

The pseudocontact shifts for the $\text{Dy} \rightarrow \text{Yb}$ complexes agree reasonably well with those calculated using the axial symmetry model and the crystal coordinates of $\text{Cr}(\text{NOTA})$ (Fig. 2). The proton and ^{13}C relaxation rates determined for three $\text{Ln}(\text{NOTA})$ complexes indicate that the smaller lanthanide cations fit into the triazamacrocyclic cavity better than do the larger ions, resulting in structurally more rigid $\text{Ln}(\text{NOTA})$ complexes.

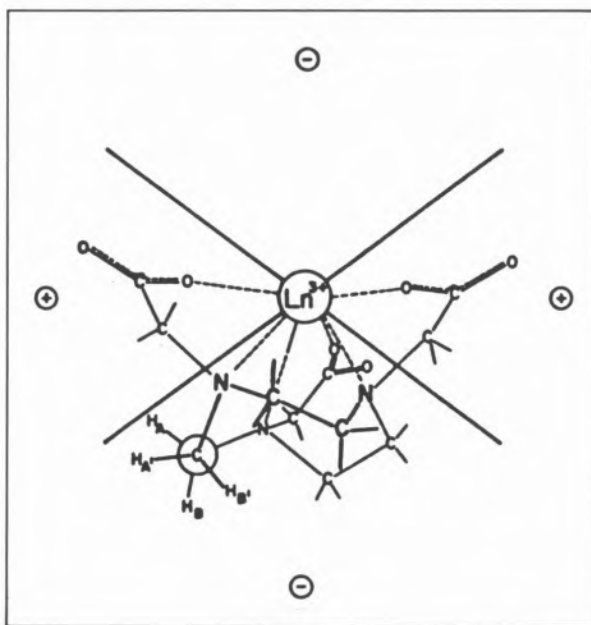


Fig. 2

The structure of $\text{Ln}(\text{NOTA})$ complexes indicating the dipolar angle and the sign of the axial dipolar geometric term

We also studied the induced LIS's of the ligand protons and ^{13}C nuclei of cyclopropane carboxylate (CPC), adenosine 5'-monophosphate (AMP) and the dicarboxylate ligand *cis*-5-norbornene-

-endo-2,3-dicarboxylic acid upon binding of the Ln(NOTA) paramagnetic complexes. Fast exchange was observed and the measured paramagnetic shifts were purified of existing contact contributions and compared with theoretical values calculated from assumed structures. The results obtained indicate that Ln(NOTA) chelates should be very useful NMR paramagnetic probes to be used in conformational analysis of small ligands or in binding to protein surfaces.

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NMRD INVESTIGATION OF Mn^{2+} AND Gd^{3+} NOTA COMPLEXES, AND A COMPARISON WITH THE ANALOGOUS EDTA COMPLEXES

It is well established that the longitudinal (spin-lattice) and transverse (spin-spin) relaxation rates of solvent protons in solutions of complexes of paramagnetic ions, *e.g.*, metalloproteins or small chelates, depend on the strength of the applied static magnetic field and on the chemical environment of the ions [1,2]. The magnetic field dependence of the proton relaxation rates of water, called nuclear magnetic relaxation dispersion (NMRD) profiles, have been previously studied in detail for complexes of Gd^{3+} and Mn^{2+} with the chelates ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) [2]. In this work, we report a study of the $1/T_1$ NMRD profiles of Gd^{3+} and Mn^{2+} ions complexed with the cyclic triazamacrocyclic ligand 1,4,7-triazacyclononane-*N,N',N''*-triacetic acid (NOTA), and compare them with results for the analogous EDTA complexes. We also discuss their potential utility as paramagnetic contrast agents in NMR imaging.

Fig. 1 shows the NMRD profiles of solvent protons of $Mn(EDTA)$ and $Mn(NOTA)$ complexes, which are independent of pH in the range of

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