multicenter bonding is responsible for the extra stabilization that leads to the observed order of dimerization energies[11,31] $\text{LiF} \sim \text{LiOH} \sim \text{LiNH}_2 \gg \text{LiCH}_3$.

The estimated $LiNH_2$ trimerization energy, about -120 kcal/mol, also is quite high and may even exceed the energy gain on $(LiF)_3$ formation (-116.8 ± 12 kcal/mol).[8] The calculated trimerization energy of $(CH_3Li)_3$ is much less, about -82 kcal/mol.[7]

The disproportion energy of $3(LiNH_2)_2 \rightarrow 2(LiNH_2)_3$ (-54.1 kcal/mol at 3-21G//3-21G) clearly favors the trimer over the dimer. The experimental observation of both dimers and trimers of $(Me_3Si)_2NLi$ can be attributed to steric effects of the bulky groups and possible solvation energy differences.

In agreement with experiment,[14] recent calculations of Raghavachari[34] on $(LiNH_2)_4$ indicate a planar eight-membered ring (D_{4h} symmetry) to be preferred over a tetrahedral arrangement (D_{2d} symmetry). The disproportion energy, $4(LiNH_2)_3 \rightarrow 3(LiNH_2)_4$, also is negative, but to a lesser extent (-25.9 kcal/mol, also at 3-21G/3-21G).

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Investigation of ^{67}Zn Coordination Effects on the ^{15}N NMR Spectra of Coordinated Ligands[1]

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A new method for the assignment of zinc(II)-nitrogen coordination schemes is reported. In this method ^{15}N NMR spectra are acquired of complexes enriched in zinc-67 and complexes at natural abundance. Resonances due to nitrogens coordinated to the zinc are broadened by scalar interaction of the nitrogen with the coordinated quadrupolar isotope while uncoordinated nitrogens show no effect from the isotopic enrichment. The method is tested with bis(diethylenetriamine) and bis(histidine) complexes. Solvent and temperature conditions are reported that repress rapid chemical exchange and allow observation of the broadening in even simple ligand systems.

The unambiguous identification of binding sites utilized by potentially polydentate ligands to coordinate to metals has been a topic of considerable interest to chemists for many years. A large group of biologically significant macromolecules are metalloenzymes that coordinate transition metals at their active site in their biologically active forms. Because of the importance of such molecules, a great many studies of the coordination details have been reported.[2-20] Within this class of compounds, zinc-containing metalloenzymes have proven to be especially difficult to study because of the lack of a good spectroscopic "handle" for the metal.

This paper reports the investigation of a new method for the assignment of coordination details in compounds containing zinc-nitrogen bonds. This method relies on the enhancement of ^{15}N T_2 relaxation by scalar coupling to a high-spin nucleus.[21] It is the purpose of this paper to demonstrate that significant ^{15}N T_2 relaxation enhancement by ^{67}Zn is observed in model compounds of known bonding. The experimental conditions under which chemical exchange is repressed and the desired behavior is observed are reported.

Experimental Section

All chemicals, except as specifically noted below, were obtained from commercial sources in the highest available purity and were used without further purification. ^{67}Zn was obtained from Oak Ridge National Laboratories as 89.68% isotopically pure zinc oxide. Diethylenetriamine was distilled at reduced pressure and stored under nitrogen in the dark until used.

All NMR spectra were recorded on a JEOL FX90Q spectrometer employing 10-mm NMR tubes. A 1.7-mm capillary containing deuterium oxide or acetone-d_6 was used for the lock signal. Neat formamide in a second capillary held alongside the lock capillary provided the ^{15}N reference signal when a nitrogen reference was desired. Temperature control was provided by the JEOL NM-VTS variable-temperature control unit calibrated with methanol.

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Metal complexes were made by mixing the appropriate molar ratio of zinc oxide and ligand with the appropriate quantity of water/glycerol solvent and adjusting the pH to the desired value with sodium hydroxide or nitric acid as required. No attempt was made to correct the pH for the effect of glycerol. Final concentrations of the zinc complexes varied from approximately 0.2 mol/L to approximately 0.5 mol/L.

Results and Discussion

Quadrupolar relaxation of a high-spin nucleus can be transmitted through a chemical bond via the scalar coupling mechanism to a spin $^1/_2$ nucleus. The efficiency of such a relaxation mechanism is a function of the magnitude of the coupling constant between the two nuclei and the relaxation time of the quadrupolar nucleus. The factors that govern the magnitude of the induced relaxation were investigated by Pople for spin 1 nuclei relaxing spin $^1/_2$ nuclei.[21] That study showed that if $T_1J \ll 0.1$, where T_1 is the spin lattice relaxation time of the quadrupolar nucleus and J is the coupling constant, insignificant relaxation of the spin $^1/_2$ nucleus is induced. Rapid chemical exchange would have a similar detrimental effect. Thus, rapid chemical exchange or spin relaxation would preclude this technique as would small values of ^1J, the one-bond ^{67}Zn–^{15}N coupling constant.

We have been unable to find literature values for the ^1J ^{67}Zn–^{15}N coupling constants or for ^{67}Zn spin lattice relaxation times in complexes of the sort of interest for bonding studies. Furthermore, zinc complexes with small ligands are known to undergo rapid chemical exchange in many cases[22-24] although exchange is believed to be slow in larger molecules for which this technique would ultimately be used.[25-27] Because it is desirable to test the technique with small molecules of known bonding, rapid chemical exchange presented a serious difficulty that had to be overcome before the feasibility of the proposed method could be tested. One can rationalize the expectation that broadening will be observed from the known ^{59}Co–^{15}N coupling constants[28,29] and zinc relaxation times in simple aqueous solution,[30] but, ultimately, experimental verification is required. It should be noted that the emphasis on broadening of the ^{15}N line requires that the nitrogen T_2 be shortened but not necessarily that the nitrogen T_1 be affected. Because T_2 relaxation has a component that is independent of Lamour frequency, T_2 shortening may be observed under conditions where T_1 is unaffected.

The first experiments performed addressed the problem of chemical exchange. A solution containing a 4:1 molar ratio of diethylenetriamine to zinc(II) was examined at probe ambient temperature (ca. 30 °C). Since diethylenetriamine is a tridentate ligand known to form a strong bis complex,[31] the nitrogen spectrum should consist of four resonances under conditions of slow exchange. Unfortunately, only two peaks are observed under these conditions indicating rapid coordinated $\rightleftharpoons$ uncoordinated exchange. In an attempt to decrease the exchange rate and nitrogen T_1 relaxation times,[32] the solvent was changed from an aqueous to ca. 30% (by volume) glycerol/water mixture. This solvent change decreased the exchange rate such that the primary nitrogen showed kinetic broadening but did not result in resolved spectra for coordinated and uncoordinated ligands. As can be seen from Figure 1, at a temperature of −3 °C the exchange rate is slowed enough that separate resonances are observed. No peaks assignable to different isomers were seen, indicating that either cis $\rightleftharpoons$ trans isomerization is rapid or the chemical shift differences between the isomers are small. Either explanation is reasonable. No indication of a meridonal isomer was observed either. By variation of the ligand to metal mole ratio the two upfield peaks were identified as the coordinated nitrogens and the downfield peaks as the uncoordinated ones. Apparent in Figure 1 is the fact that the primary nitrogens are shifted considerably more upon coordination than is the secondary. Clearly, though, because the shifts introduced by coordination are small (ca. 14 ppm for the primary nitrogen and ca. 3 ppm for the secondary nitrogen) compared to protonation shifts, one would have great difficulty trying to assign coordination details in a complex ligand on the basis of chemical shift difference between coordinated and uncoordinated ligands. Indeed, if coordination resulted in a proton transfer to an uncoordinated nitrogen, the shift upon coordination of an uncoordinated nitrogen may be greater than a coordinated one! Thus, one must be very careful when attempting to use ^{15}N shifts to assign coordination details in complex molecules. The limited data reported here are consistent with very small coordination shifts.

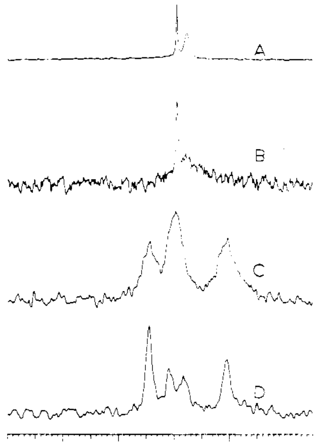

Figure 1. Natural-abundance ^{15}N spectra of 4:1 molar ratio dien:Zn(II) in 30% glycerol/water solvent (Zn(II) concentration, approximately 0.5 mol/L; pH 8.06; points; 500-Hz scan; 90° pulse width; 0.4-s repetition rate): A, 30 °C; B, 12 °C; C, 2 °C; D, −3 °C. Each spectrum consists of approximately 23 300 acquisitions under conditions of complete proton decoupling.

The data summarized in Figure 1 were used to calculate the approximate exchange rate at several temperature from the coalescence of the nitrogen resonances. These data lead to activation parameters of 51 kJ/mol and −10 J/mol K for ΔH and ΔS, respectively. These values predict that the rate at 25 °C should be approximately 2×10^3 s^{-1}. This predicted rate is approximately equal to the reported exchange rate for glycine at 25 °C (1.4×10^3).[24] Because of the limited temperature range accessible and the significant experimental uncertainty of the measurements, these rates should be viewed as rough estimates rather than as extremely accurate kinetic parameters. Nevertheless, they strongly indicate that the experimental conditions described above should lead to slow chemical exchange in even simple ligand systems. Although

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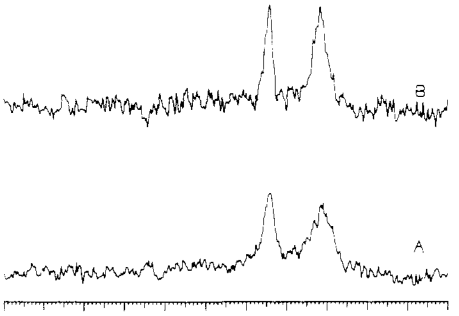

Figure 2. Natural-abundance ^{15}N spectra of 2:1 molar ratio dien:Zn(II) in 30% glycerol/water solvent at −2 °C (zinc concentration, approximately 0.5 mol/L; acquisition parameters same as in Figure 1): A, natural-abundance zinc; B, ^{67}Zn enriched.

no large ligand studies are reported in this paper, it is to be expected that the exchange rates of larger ligands will be slower than these simple ones and, hence, the conditions reported here (or warmer) should be suitable for virtually all zinc(II) complexes.

Figure 2 shows the results of an enrichment study of the bis(diethylenetriamine)zinc(II) complex. Examination of Figure 2 shows observable although modest broadening upon isotopic enrichment. At −2 °C the primary nitrogens have been broadened from ca. 21.5 Hz to ca. 28.3 Hz while the secondary nitrogens have been broadened from 10.7 to 13.7 Hz. That both resonances are broadened is consistent with the fact that all of the ligand nitrogens coordinate. A similar study at 0 °C produced coordination broadening of 5 Hz on the secondary nitrogen and 10 Hz on the primary nitrogens. The uncertainty in the measured broadening in both of these studies is approximately ±3 Hz as estimated by the procedure explained below.

In view of the relatively slight broadening observed for a symmetrical molecule, one might wonder if any broadening at all will be observed for a less symmetrical one. To test such a case, the bis(histidine) complex was investigated. The results of this study are shown in Figure 3.

Histidine has three nitrogens, only two of which can coordinate. The coordination of histidine to zinc(II) is known to involve the NH_2 and π N with the τ uncoordinated.[34,35] This ligand represents a crucial test of the proposed method since it contains both coordinated and uncoordinate nitrogens. If the method is a viable one, broadening of both coordinated nitrogens must be observed while, equally importantly, no broadening of the uncoordinated nitrogen should occur. Examination of Figure 3 shows the desired behavior. The peak widths at half-height measured from the raw data shown in Figure 3 (τ, π, and NH_2, respectively) are as follows: 4.6, 18.3, and 4.3 Hz (natural abundance); 4.3, 43, and 9.8 Hz (^{67}Zn). The experimental uncertainty of each measurement is approximately ±1 Hz except for the π measurements for which the uncertainty is estimated as approximately ±2 Hz in the unenriched sample and ±8 Hz in the enriched sample. These uncertainties were estimated by assuming that the error in peak height was equal to $\pm^1/_2$ of the peak-to-peak noise level and measuring the uncertainty in half-width that would result from such an error in height. This procedure appears to overestimate the uncertainty as the observed reproducibility of half-width has been better than this criterion would predict. The above observations are precisely the desired behavior and indicate that this technique is a useful one for coordinate assignments even in the case of relatively nonsymmetrical ligands such as histidine.

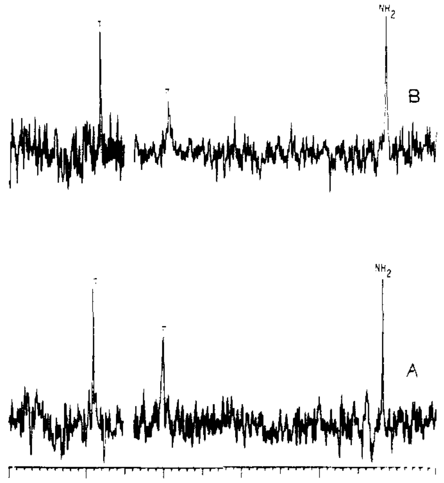

Figure 3. Natural-abundance ^{15}N spectra of 2:1 molar ratio histidine:zinc(II) in 30% glycerol/water (16 °C; zinc concentration, approximately 0.2 mol/L): A, natural-abundance zinc; B, ^{67}Zn enriched. τ-Nitrogen spectrum was obtained by the Ernst/Doddrell sequence adjusted to estimated ^2J ^{15}N–^1H coupling constant (spectral parameters: 4K data points; 2500-Hz scan; 30 m/s Δ and τ delays; approximately 25 000 scans; repetition rate 1.5 s). NH_2 and π spectra were obtained by single-pulse experiment (spectral parameters: 8K data points; 2500-Hz scan; 30° pulse; approximately 114 000 scans; repetition rate 0.82 s).

The application of this technique to large ligand systems such as zinc metalloenzymes will require extremely long data acquisition times at the field strength and sample size employed here. A high-field wide-bore instrument and/or nitrogen-15 enrichment is recommended for large-molecule studies. However, these measurements should not require low temperatures or highly viscous solvents as were required for this study of small molecules.

Registry No. ^{67}Zn, 14378-34-8; bis(histidine)zinc(II), 16743-16-1; bis(diethylenetriamine)zinc(II), 46358-79-6.

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