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Cadmium-113 Magnetic Resonance Spectroscopy

Paul D. Ellis

Summary. Cadmium-113 nuclear magnetic resonance spectroscopy has been used in studies of the structure and dynamics of inorganic and bioinorganic molecules. Chemical dynamics play an important role in the analysis of relaxation and chemical shift data. Naïve interpretations of relaxation data can be checked by performing these experiments at a variety of temperatures and magnetic field strengths. A combination of solid- and liquid-state nuclear magnetic resonance measurements can provide the user with unambiguous data on chemical shielding. These data can be used to characterize zinc and calcium ion binding sites in metalloproteins.

We have witnessed a large increase in the number of applications of nuclear magnetic resonance (NMR) methods to research problems in physics, chemistry, and biology. One of the many factors responsible for this increase is the routine acquisition of NMR data from virtually any nucleus in the periodic table. This application of NMR methods to the study of spin-½ metal nuclides and the interpretation of the data obtained are the subjects of this article. The examples used come from experience in using ^{113}Cd NMR spectroscopy to investigate systems of bioinorganic interest. Even though the applications involve ^{113}Cd NMR, the principles are applicable to any spin-½ metal nuclide. However, there are pitfalls in extracting chemical information from multinuclear NMR data. These pitfalls generally arise when one ignores the basic chemistry and physics that differentiate a metal from a lighter element such as carbon.

Cadmium NMR Spectral Parameters

Sensitivity. The ^{113}Cd resonance of 0.1*M* aqueous $Cd(ClO_4)_2$ solution is shown in Fig. 1. Compared to the routinely observed natural abundance ^{13}C nucleus, the signal from ^{113}Cd is 7.6 times as intense. This comparison takes into account the responses of the carbon and cadmium isotopes to a given magnetic field strength and their relative natural abundance (*1*).

In certain cases carbon and cadmium cannot be easily compared. Carbon-13 is a spin-½ nucleus that often has dipole coupling to abundant hydrogen nuclei. In such a situation the nuclear Overhauser effect (*2, 3*) can be used to enhance the carbon signal by a factor as large as 3.98. Cadmium-113 is a negative spin-½ nucleus and the Overhauser effect generally leads to a decrease in cadmium signal intensity.

In biological applications of NMR spectroscopy the concentrations usually dictate the use of enriched isotopes, and hence ^{113}Cd and ^{13}C are similar in sensitivity. The NMR parameters of several spin-½ metal nuclides are summarized in Table 1. If we consider that experiments within ^{13}C solid and liquid states are routine, then for all the nuclides summarized in Table 1 except ^{57}Fe and ^{103}Rh, the multinuclear NMR experiment should be comparable in difficulty to a ^{13}C experiment. For practical reasons the apparent lower concentration limit for enriched ^{13}C is 0.1 to 0.05 m*M*. There are several factors that can alter these numbers (line widths, magnetic field strength, and relaxation times), but this limit is a reasonable guide.

One consequence of the favorable sensitivity of ^{113}Cd is the use of cadmium as an NMR probe. Cadmium can replace ions such as calcium or zinc in molecules of biological origin. Figure 1b depicts the ^{113}Cd NMR spectra of skeletal troponin C (STnC), cadmium having replaced calcium in all structural and regulatory binding sites. Clearly, detailed information about metalloproteins can be obtained from cadmium NMR spectra by using commercially available instruments and reasonable data acquisition periods. In recent years this high sensitivity has been utilized in a number of studies of cadmium in inorganic and organometallic compounds. To properly appreciate the possible applications of ^{113}Cd NMR spectroscopy, one needs a firm understanding of the chemical shift parameters in a variety of ligand environments.

Chemical shifts. Figure 2 presents a schematic overview of chemical shifts for ^{113}Cd similar to the one prepared by Haberkorn *et al.* (*4*). The uppermost portion depicts the chemical shift range of cadmium with oxygen ligands, as determined by Maciel and Borzo (*5*), Kostelnik and Bothner-by (*6*), and Cardin *et al.* (*7*). Ohtaki *et al.* (*8*), using x-ray diffraction, determined the solution structure of $Cd(H_2O)_6^{2+}$ to be octahedral. Methanol, dimethylformamide, and dimethyl sulfoxide (DMSO) solvents also yield six-coordinate complexes with "oxygen type" ligands whose cadmium chemical shifts fall in the range indicated here (*8–10*).

The linear concentration dependence (*6*) of the inorganic cadmium salts in aqueous solution could be explained by inner- or outer-sphere complexation with

The author is professor of chemistry and director of the NSF-sponsored NMR Regional Instrumentation Facility at the University of South Carolina, Columbia 29208.

Table 1. Properties of spin-½ metals relative to ^{13}C.

Nucleus	Resonance frequency (MHz) at 9.4 T (400 MHz for ^1H)	Natural abundance (%)	γ [(Hz · G) $\times 10^{-3}$]	Relative sensitivity for equal number of spins
^{13}C	100.62	1.108	1.075	1.0
^{57}Fe	12.928	2.245	0.1376	2.13×10^{-3}
^{103}Rh	12.693	100.0	0.1351	1.96×10^{-3}
^{109}Ag	18.622	48.65	0.1982	6.35×10^{-3}
^{113}Cd	88.756	12.34	−0.9454	0.685
^{119}Sn	149.211	8.68	1.5878	3.25
^{195}Pt	85.832	33.7	0.9134	0.625
^{199}Hg	77.568	16.86	0.7616	0.360
^{207}Pb	83.599	21.11	0.8896	0.574

the given anion (*4*). At "infinite dilution," these cadmium resonances approach that for 0.1*M* aqueous cadmium perchlorate, the generally accepted standard for cadmium chemical shift measurements.

The next section in Fig. 2 shows the chemical shift range of cadmium-halide complexes in solvents that provide oxygen ligands. The shift of CdX_2 is a distinctly nonlinear function of concentration (*6*). Addition of alkali metal halides (*11*) or ammonium halides (*12, 13*) to solutions of cadmium salts results in deshielding in the order CdX_3^-, CdX_2, CdX^+. It is not known whether this dependence is the result of ligand rearrangement (octahedral ⇄ tetrahedral) or ligand type (oxygen ⇄ halogen), although it appears that the most shielded resonances are those with the greatest number of oxygen ligands.

The next shift range in Fig. 2 is that for cadmium with carbon ligands. The most deshielded resonance is that for dimethyl cadmium in the nonassociating solvent cyclohexane. In solvents that provide some ligation through oxygen or nitrogen, the cadmium may be shielded by as much as 100 ppm (*7*). Alkyl ligands other than methyl cause shielding with respect to dimethyl cadmium (*7*), diphenyl cadmium in dioxane solvent being the most extreme [more shielded than $(CH_3)_2Cd$ by 314 ppm]. Approximately 50 ppm of this chemical shift could arise from the dioxane solvent. The oxygen of the methyl cadmium alkoxides appears to cause appreciable shielding of cadmium (*14*). These compounds can self-associate in solution to form dimers [such as $(CH_3CdO\text{-}t\text{-}C_4H_9)_2$:benzene], tetramers [$(CH_3CdOC_2H_5)_4$:benzene], and hexamers [$(CH_3CdS\text{-}i\text{-}C_3H_7)_6$] (*15*). The cadmium-thiolate complexes exhibit the greatest known shifts to lower shielding (*16*). Magnetic circular dichroism indicates near-tetrahedral symmetry about cadmium, and it is suggested that the greatest deshielding is from total sulfur ligation, the aqueous solvent being completely excluded. The thiophenol complex $[Cd(SC_6H_5)_4]^{2-}$, observed by Carson *et al.* (*16*), produces the least shielded cadmium resonance (δ = 829 ppm).

The remaining 60 percent of this chemical shift scale includes the mixed ligand complexes Cd-S,Se and Cd-S,O, the heteroligands causing higher shielding. The selenium ligands, studied by Carson and Dean (*17*) and Dean (*18*) for their ability to moderate the extreme toxicity of cadmium, shield cadmium slightly ($\Delta\sigma \approx 30$ ppm) with respect to sulfur. Mixed (sulfur and oxygen) ligands are capable of shielding of several hundred parts per million relative to tetrahedral CdS_4 (*4, 19*).

An example of pure cadmium-nitrogen ligation is the pyridine adduct of cadmium-tetraphenylporphyrin (Cd-TPP:Pyr) in chloroform solvent (*20*), the cadmium resonance occurring at 436 ppm. Modification of the pyridine adduct induces small changes in the cadmium chemical shifts (*21*). Strong shielding is provided by the mixed nitrogen-oxygen ligands of aqueous cadmium-ethylenediaminetetraacetic acid (Cd-EDTA) (*22*) and cadmium-benzoyltrifluoroacetone [Cd-$(BTA)_2$] in pyridine (*23*). Studies of polydentate amine ligands (*24*) reveal bridged compounds with octahedral symmetry about cadmium in the solid state. The mixed Cd-(N,S,O) complex di-μ-chlorodichlorobis-(6-mercaptopurine) diaquodicadmium(II) [Cd-$(CDM)_2$] was studied in DMSO-d_6 by ^{113}Cd NMR and in the solid state by x-ray diffraction (*25*). Dimers are found in both solution and crystal with octahedral symmetry about cadmium. In DMSO the cadmium resonance is 554 ppm deshielded from Cd-TPP:Pyr, illustrating the greater deshielding ability of sulfur ligands relative to the nitrogen and oxygen.

The final chemical shift scale in Fig. 2 is of mixed-ligand cadmium-phosphine complexes. The experiments were performed at low temperature in dichloromethane to slow the chemical exchange of phosphine and anions (*26, 27*). Consistent with previous examples, adducts with oxygen ligands (such as NO_3^-) are most shielded ($\delta \approx 100$ ppm). Shifts to higher shielding are also seen for the cadmium dimers that typically form when only one phosphine per cadmium is present. These 1:1 adducts contain cadmium in distorted tetrahedral sites (*26*). The monomeric 1:2 adduct $CdX_2(PR_3)_2$ (R, alkyl) is also tetrahedral about cadmium and represents the most deshielded resonance (*27*).

The chemical shifts observed for cadmium-substituted proteins (Fig. 3) are qualitatively consistent with the general trends found in nonbiological systems. The binding sites containing sulfur ligands, such as metallothioneine (*28*) and liver alcohol dehydrogenase (*29–34*), are the least shielded ($\delta \approx 600$ ppm), while sites with only oxygen ligands, such as concanavalin A (Con A) (*35, 36*) and parvalbumin (*36, 37*), are the most shielded ($\delta \approx -100$ ppm). A more general discussion of ^{113}Cd chemical shifts is presented elsewhere (*38*).

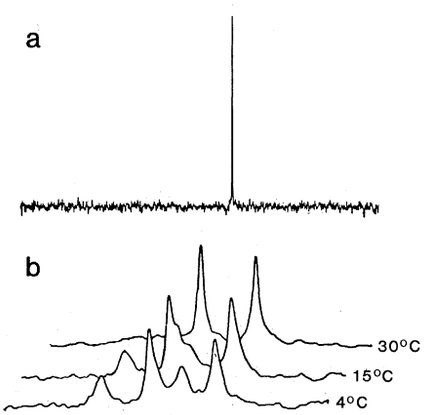

Fig. 1. Natural abundance ^{113}Cd NMR spectra obtained at 9.4 T (88 MHz). (a) Spectrum obtained with a single 90° pulse from 0.1*M* aqueous cadmium perchlorate. The line width is 0.4 Hz and the signal-to-noise ratio is 38:1. (b) Spectra obtained from cadmium-substituted STnC at the temperatures indicated. A signal-to-noise ratio of 30 was achieved with 4½ hours of accumulation time (40,000 transients) with 2.5 ml of 2.5 m*M* protein.

Interpretation of Metal Nuclide Chemical Shifts

Chemical dynamics. From the previous section it should be clear that ^{113}Cd chemical shifts are sensitive to subtle structural changes. However, associated with this sensitivity is the potential caveat with respect to the time scale for

ligand dynamics. Such a situation can bring about intermediate or rapid exchange between the various ligand environments, and the observed spectrum becomes the result of an average over all the ligand sites.

One of the experimental facts of life that plagues the practitioner of spin-½ metal NMR spectroscopy is the disappearance of NMR signals. This larceny often arises from unforeseen dynamics. If the experimental system is biological then the solution to this problem is simple but frustrating: find another system. The reason for such a decision is simple. If the origin of the problem lies in dynamics, then the biological system resists low-temperature experiments because of the necessity of an aqueous solution.

The problem of unfortunate dynamic time scales has been solved by Ackerman and Ackerman (*39*) in a procedure based on the methods of Rasmussen and Mackenzie (*40*) and Turnbull (*41*) for supercooling aqueous solutions. Ackerman and Ackerman obtained the ^{113}Cd NMR spectrum of 0.1*M* $Cd(ClO_4)_2$ in the presence of various amounts of glycine. By varying the concentration of the glycine and the solution *p*H, they were able to observe separate resonances for what appeared to be $CdGly^+$, $CdGly_2$, $CdGly_3^-$, and $CdGly_4^{2-}$. The tentative assignment of these resonances was confirmed by Jakobsen and Ellis (*42*) who, using the above procedure with ^{15}N-enriched glycine, observed the cadmium-nitrogen coupling pattern expected for the proposed complexes.

It is not easy to recognize when chemical dynamics are present in a particular system. For example, the ^{113}Cd NMR spectra of $(CH_3)_2Cd$ and $(C_2H_5)_2Cd$ yield sharp resonances with well-defined proton-cadmium and carbon-cadmium coupling constants (*7*). It is not until one mixes these two compounds and obtains the ^{113}Cd NMR spectrum of the mixture that the ligand exchange processes become obvious. One observes three resonances: $(CH_3)_2Cd$, $(CH_3)Cd(C_2H_5)$, and $(C_2H_5)_2Cd$ (*7*).

In the preceding organometallic system the ligand exchange became a novel example of how one could study such processes. However, as Jensen *et al.* (*22*) found, in the Cd-EDTA system ligand exchange processes represent a serious pitfall. In this case the *p*H dependence of the ^{113}Cd relaxation time T_1 in Cd-EDTA was examined. At *p*H values above 11 there was a precipitous de-

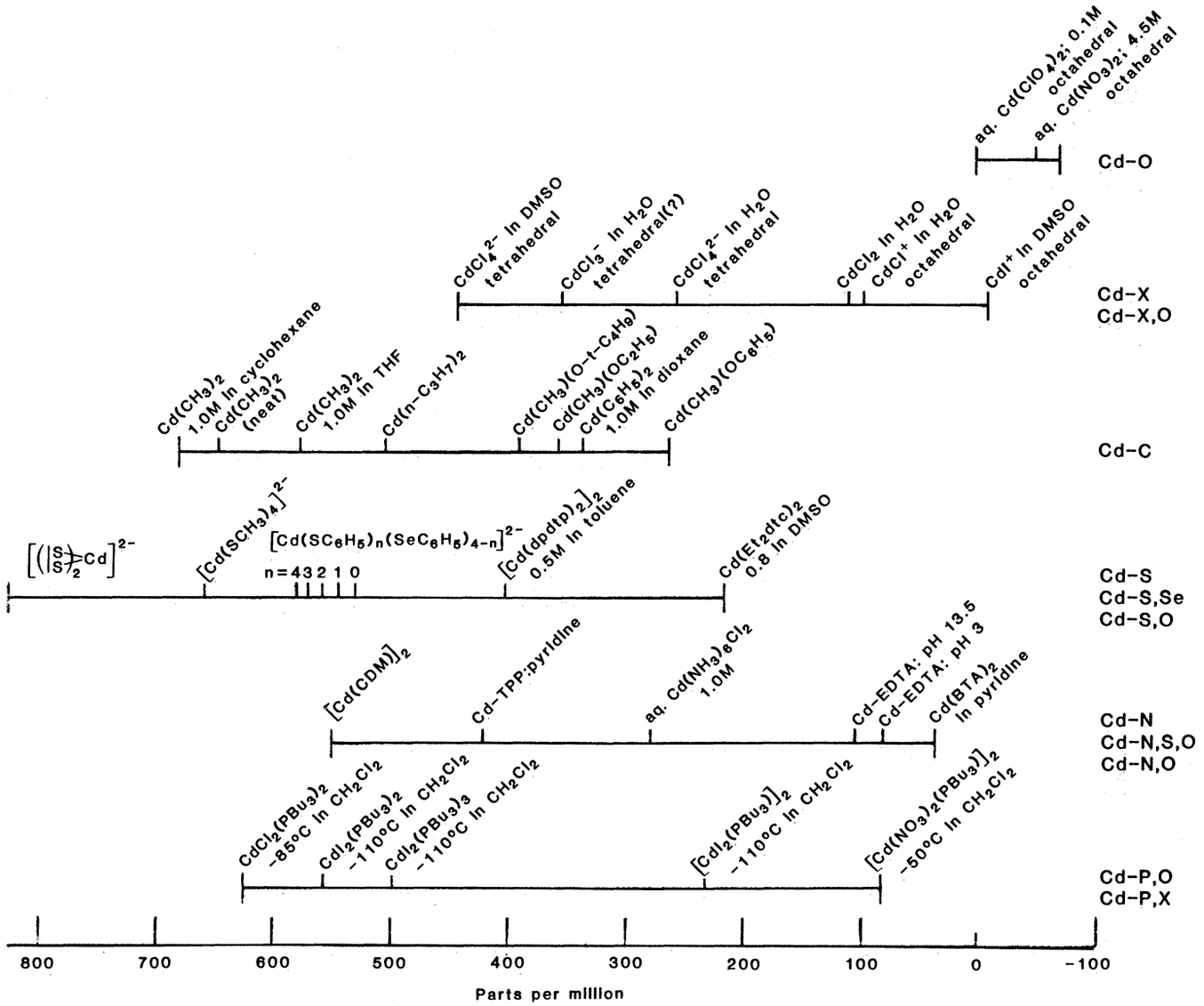

Fig. 2. Chemical shift ranges for various cadmium-containing compounds. The horizontal scale is cadmium chemical shift in parts per million referred to 0.1*M* aqueous $Cd(ClO_4)_2$. Positive values of the chemical shift indicate resonances to lower shielding. Abbreviations: *DMSO*, dimethyl sulfoxide; *THF*, tetrahydrofuran; *dpdtp*, *O,O'*-diisopropyl dithiophosphinato; *Et₂dtc*, *N,N'*-diethyldithiocarbamato; *[Cd(CDM)]₂*, di-μ-chloro-dichlorobis-(6-mercaptopurine) diaquodicadmium(II); *PBu₃*, tributyl phosphine; and *TPP*, tetraphenylporphyrin.

crease in the ^{113}Cd T_1. After an extensive analysis of the field dependence of the ^{113}Cd T_1 it was concluded that this sudden drop in T_1 resulted from a rapid chemical exchange between Cd-EDTA and, in all probability, a hydroxylated form of Cd-EDTA. From the relaxation data it was concluded that this latter species had a much shorter T_1 and hence that the measured T_1 was reflecting the exchange dynamics to the relaxation sink and not the spin lattice relaxation properties of the system of interest. Hence, if it is not known that chemical exchange processes are operational, incorrect conclusions can be reached about the importance of various relaxation mechanisms.

Ellis *et al.* (*43*) extended this analysis to a three-site exchange network, cadmium-substituted Con A. By employing double-saturation transfer techniques they were able to deduce the exchange contributions to the ^{113}Cd T_1's. It is essential to know whether chemical exchange is present before one draws conclusions about the relative importance of the various relaxation mechanisms or the presence of internal motions.

Complementary nature of solid- and liquid-state NMR. It may be possible to use the sensitivity of the ^{113}Cd chemical shift to structural parameters to probe the details of the metal binding site. What factors give rise to the ^{113}Cd chemical shift? Are these factors easily translated into such terms as ligand donor or acceptor properties, bond distances, coordination number, and overall complex topology? To unambiguously address these questions, our experimental data must be free of the effects of chemical dynamics. Hence, we will employ chemical shifts determined by solid-state methods. This approach has the further advantage that one can correlate these data with well-defined structures obtainable by standard x-ray methods.

The magnitude and sign of substituent effects on ^{13}C chemical shifts were found to be consistent with our "chemical common sense" regarding inductive and resonance effects (*44*). For example, the substituent effects caused by halogens, $\delta_{CH_3I} < \delta_{CH_3Br} < \delta_{CH_3Cl} < \delta_{CH_3F}$, was considered normal. In Table 2 are summarized some solid-state ^{113}Cd chemical shifts measured by Nolle (*45*) for CdI_2, CdF_2, $CdBr_2$, $CdCl_2$, CdO, and CdS. It is clear from these data that halogen substituent effects cannot be considered "normal" or "inverse" with respect to the order of the ^{13}C chemical shifts. Other factors influence these chemical shifts. Is it reasonable to assume that metal nuclides such as cadmium should share similar patterns of chemical shielding with nuclei such as carbon? The answer is no. The fundamental reason for this lies in the concept of spin-orbit couplings.

Table 2. Aberrant ^{113}Cd chemical shifts (neither "normal" or "inverse" halogen effects).

Compound	Chemical shift (*60*)
CdI_2	0.0
CdF_2	449
$CdBr_2 \cdot 4H_2O$	713
$CdCl_2$	883
CdO	1091
CdS	1222

Atomic spectroscopists have long known that selection rules based on the quantum numbers **L** and **S** progressively break down as the atomic number increases beyond 35 (Br) (*46*). This difficulty arises because of spin-orbit effects, $\mathbf{L} \cdot \mathbf{S}$. An electron in a nonspherically symmetric orbital is equivalent to a current. This current produces a magnetic field that interacts with the spin of the electron and produces a change in energy in somewhat the same way as the interaction of a current with an applied magnetic field. Inclusion of the spin-orbit interaction term in the Hamiltonian for molecules invalidates the concept of the spin molecular orbital, since the presence of the operators $\mathbf{S}_x$ and $\mathbf{S}_y$ will lead to a mixing of α and β spin character into a single molecular orbital. This introduces the concept of nonperfect pairing. The effect is small, but it influences chemical shifts and coupling constants. It occurs in the absence of an applied magnetic field and, for a closed-shell diamagnetic molecule, has the effect of mixing triplet character into the singlet ground state. In the presence of an applied magnetic field the triplet character results in a spatial imbalance of α and β spin character of the electrons, which results in an induced spin density throughout the molecule. The resulting spin density generates a local field at a given nucleus. These local fields are transmitted throughout the molecule by mechanisms that are analogous to those for the indirect nuclear spin-spin coupling constant. I do not intend to present a detailed theoretical account of spin-orbit effects on chemical shifts (*47*) but rather point out that such effects are important for a proper discussion of metal nuclide chemical shifts and spin coupling constants.

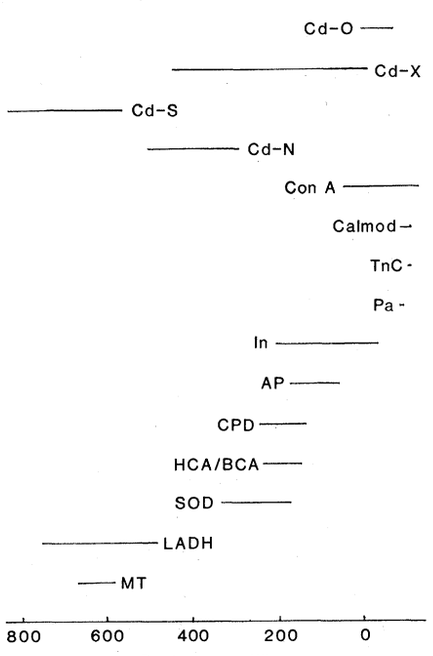

Fig. 3. Chemical shift ranges of cadmium metalloproteins, compared with chemical shifts determined for cadmium complexes. Abbreviations: *Con A*, concanavalin A (*35, 36*); *HCA*, human carbonic anhydrase (*32*); *Pa*, parvalbumin (*37, 53*); *BCA*, bovine carbonic anhydrase (*30, 32*); *TnC*, troponin C (*56, 57*); *AP*, alkaline phosphatase (*32*); *Calmod*, calmodulin (*58*); *SOD*, superoxide dismutase (*33*); *In*, insulin (*67*); *LADH*, liver alcohol dehydrogenase (*34*); *CPD*, carboxypeptidase A (*55*); and *MT*, metallothioneine (*28*).

As an example of how solid-state NMR data can be used to analyze chemical shifts, consider Cd-TPP:Pyr. Line shapes of NMR powder patterns are well understood and have been the subject of several reviews (*48*). From the prominent features of these spectra, such as those in Fig. 4, it is often possible to derive the principal components of the shielding tensor (σ_{xx}, σ_{yy}, σ_{zz}) directly. For a system with axial symmetry $\sigma_{xx} = \sigma_{yy} = \sigma_\perp$ and $\sigma_{zz} = \sigma_{||}$. Here $\sigma_\perp$ and $\sigma_{||}$ refer to tensor elements that are perpendicular or parallel to the threefold or higher axis of rotation. The isotropic chemical shift is the trace of the shielding tensor, $\sigma_{iso} = (\sigma_{xx} + \sigma_{yy} + \sigma_{zz})/3$.

The isotropic shift of 432 ppm observed for Cd-TPP:Pyr is similar to that observed from liquid-state NMR with $CHCl_3$ as the solvent. Furthermore, the solid-state isotropic shift data in Fig. 4 show that addition of pyridine as a fifth ligand to Cd-TPP shifts the ^{113}Cd resonance to lower shielding by 33 ppm. The power of solid-state NMR spectroscopy is, however, best appreciated when it is realized that this relatively small isotropic chemical shift difference is the result of large changes in the individual shielding tensor elements moving in the opposite directions. That is, the unique tensor

element ($\sigma_{\parallel}$) for Cd-TPP:Pyr is shifted to higher shielding by 124 ± 5 ppm while the in-plane element ($\sigma_{\perp}$) is shifted to lower shielding by 112 ± 5 ppm relative to Cd-TPP. From these changes in shift tensor elements, the structural factors that give rise to the observed isotropic chemical shift of 33 ppm may be argued qualitatively, an argument that would have been meaningless without the solid-state NMR information (*49–51*).

Our knowledge of ^{113}Cd chemical shifts is based solely on experimental observations. Until a general theoretical picture emerges for chemical shifts of heavier elements, one must rely on a close empirical relation between the benchmarks of solid-state chemical shift data and well-known structures. Here, chemical shift does not mean only the isotropic chemical shift but also the elements of the cadmium chemical shift tensor.

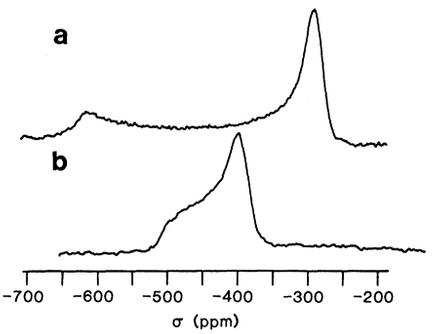

Fig. 4. Cross-polarization solid-state ^{113}Cd NMR powder spectra of (a) "free" Cd-TPP and (b) the [^{15}N]pyridine adduct of Cd-TPP. The shielding constant (σ) scale is positive for larger shieldings and is referred to a sample of solid $Cd(ClO_4)_2 \cdot 6H_2O$.

Cadmium-113 NMR Spectroscopy of Skeletal Troponin C

One of the principal motivations behind efforts with ^{113}Cd NMR spectroscopy has been the desire to develop a clean spectroscopic probe for zinc and calcium in biological systems. The first observation of ^{113}Cd signals from a metalloprotein was made by the Yale group headed by Armitage and Coleman (*32, 52*). Other ^{113}Cd observations quickly followed (*31, 34, 36, 53*). Rather than present an extensive review of this work, I will focus on recent research on the calcium-binding muscle protein STnC (*54–56*).

Forsèn and co-workers have been leaders in applying ^{113}Cd NMR to calcium-binding proteins, parvalbumin (*37, 53*), STnC (*57*), and calmodulin (*58*). With parvalbumin, they were able to observe resolvable resonances for each of the high-affinity Ca^{2+} binding sites. However, with STnC they were only able to observe the resonances for the structural sites.

As important as these pioneering experiments were, they cast doubt on the utility of using ^{113}Cd NMR spectroscopy as a calcium probe in these systems. However, the group headed by J. D. Potter and me has observed resonances for all four calcium binding sites in STnC. By a series of Cd^{2+} and Ca^{2+} titrations they have assigned the resonances to two classes of binding sites. Their assignment of the structural sites is partially consistent with that reported by Forsèn *et al.* (*57*).

The binding of cadmium to STnC has been followed by equilibrium binding studies and by ^{113}Cd NMR spectroscopy (*54*). The equilibrium binding experiments showed that there are two cadmium binding sites on STnC with a high affinity for Cd^{2+} ($K_{Cd} \approx 10^7 M^{-1}$) and two with a lower affinity for Cd^{2+} ($K_{Cd} \approx 10^3 M^{-1}$). The former binding constant is comparable to Ca^{2+} binding to the Ca^{2+}-Mg^{2+} (structural) sites and the latter is about 100 times less than Ca^{2+} binding to the Ca^{2+}-specific (regulatory) sites.

The ^{113}Cd NMR was shown to be temperature-dependent. The room temperature spectrum consists of two resonances at −107.8 and −112.7 ppm with respect to a 0.1*M* solution of $Cd(ClO_4)_2$. Lowering the temperature to 4°C alters the cadmium exchange dynamics and results in a four-line ^{113}Cd spectrum. The two new resonances at −103.1 and −109.8 ppm arise from cadmium binding to the Mg^{2+} sites on STnC, whereas the resonances at −107.8 and −112.7 ppm correspond to cadmium binding at Ca^{2+} regulatory and structural sites, respectively. The NMR data cannot distinguish between ordered binding to the Ca^{2+}-Mg^{2+} sites (one metal site being occupied at a time) and random binding to a particular class of binding site. Further, an allosteric coupling network exists between all classes of binding sites. This coupling may be important physiologically, depending on whether Ca^{2+} or Mg^{2+} is bound to the Ca^{2+}-Mg^{2+} sites (*56*).

The isotropic chemical shifts for the calcium-binding proteins fall in a characteristic range of chemical shifts, −85 to −130 ppm (*59*). The inability to produce model compounds with chemical shifts in this region must be due to ligand dynamic processes that are present in the model compounds but absent in the proteins. To circumvent the problems associated with ligand dynamics in the solution phase, many researchers (*11, 20, 45, 60–62*) have studied the ^{113}Cd chemical shifts (both isotropic and the shielding tensors) of cadmium-oxo compounds in the solid state. The only pattern that has emerged from these observations is that six-coordinate cadmium has a range of ^{113}Cd chemical shifts from 150 to −60 ppm, seven-coordinate cadmium spans a range from 0 to −60 ppm, and eight-coordinate cadmium can be observed from 0 to −100 ppm.

From the solid-state ^{113}Cd NMR data one would argue that the isotropic chemical shifts observed for STnC, calmodulin, parvalbumin, and the S2 site in Con A are representative of eight-coordinate cadmium and not six-coordinate cadmium. In this light it is interesting to note that Kretsinger, in his discussion of the structure of carp muscle parvalbumin (*63*) and in his subsequent review of calcium-binding proteins (*64*), pointed out that the so-called EF calcium site in parvalbumin is eight-coordinate and that the CD site is six-coordinate. From the preceding arguments one would have to conclude that both sites in the cadmium-substituted protein in solution are eight-coordinate.

Tufty and Kretsinger (*65*) proposed, after an incisive review of the homologies in amino acid sequence between parvalbumin and STnC, that STnC consists of four EF hands arranged in two pairs. I agree, since the EF site is eight-coordinate in parvalbumin and the isotropic ^{113}Cd chemical shift of STnC ranges from −103 to −113 ppm. The data strongly support the idea that all the cadmiums and hence the calciums reside in eight-coordinate metal binding sites. However, it is equally clear that more solid-state ^{113}Cd NMR data on both model compounds and on the proteins themselves and more refined x-ray data are needed before any firm conclusions can be drawn.

Summary and Conclusions

In this review I have endeavored to demonstrate the utility of ^{113}Cd NMR spectroscopy in bioinorganic and inorganic chemistry. Although, as with any spectroscopic technique, ^{113}Cd NMR has its pitfalls, it can be used to address important dynamic problems and to provide essential data on chemical rate constants and NMR relaxation times. With solid-state NMR techniques one can study subtle changes in structure and bonding through the sensitivity of the

shielding tensor. Further, knowledge of these shielding tensors can then be used in liquid-state NMR experiments to follow anisotropic motion in important bioinorganic systems. Ellis and co-workers (*35, 54*) highlighted this in their study of the chemical dynamics of Con A and in their demonstration of allosteric coupling in STnC, respectively.

Although the specific examples in this article have been limited to ^{113}Cd, the conclusions apply to other spin-½ nuclides. In the context defined by their chemistry, all spin-½ metal nuclides hold promise as molecular structure probes for research problems in bioinorganic and inorganic chemistry.

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