Unusual Slow-Exchange ^{113}Cd NMR Spectra Observed at Ambient Temperature for Halide, Cyanide, and Mercaptide Cadmium Coordination Compounds with Benzimidazole Ligands. Applications to Cd-Substituted Metalloproteins

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Abstract: Slow-exchange ^{113}Cd NMR spectral data ($Cd + L \rightleftharpoons Cd(L)$) were obtained for ligands (L) containing benzimidazole donors in Me_2SO-d_6 solution and at ambient temperature. One compound, Cd(L1) (L1 = $(BzCH_2)_3N$; Bz = 2-substituted benzimidazole), formed new derivatives (Cd(L1)X) which also exhibited slow-exchange ^{113}Cd NMR spectra ($Cd(L1) + X \rightleftharpoons Cd(L1)X$) on addition of the anions (X) Cl^-, Br^-, I^-, CN^-, PhS^-, and L-cysteine$^-$. Typically the ^{113}Cd shift of Cd(L1)X was ~170 to ~300 ppm to higher frequency to that of Cd(L1). These are the first examples of slow-exchange solution spectra at ambient temperature which result from the binding of a unidentate anion in a small Cd coordination compound. These novel findings allowed the quantitative determination of the influence of these anions on the ^{113}Cd NMR chemical shift. The relatively slow exchange of X in Cd(L1)X species, when compared to other Cd(L)X species, is discussed in terms of a "pocket" formed by the L1 ligand which inhibits bimolecular exchange processes and allows the study of ^{113}Cd NMR in a model enzyme environment. The chemical shift information obtained was used to analyze the chemical shift results reported for Cd-substituted metalloproteins containing N and O donors or N, O, and one X donor at the metal binding site. Except in a few cases, the shifts of Cd occupying the metal binding site of zinc and copper proteins could be interpreted with reasonable accuracy. Discrepancies between reported experimental shift data for some proteins are discussed in terms of anion binding effects. Contrary to earlier interpretations, it seems likely that Cd(II), in some cases, coordinates to a different number of non-oxygen donor ligands than are coordinated to the metal in the native protein.

The potential and versatility of ^{113}Cd NMR spectroscopy as a probe of metal ligation sites in metalloproteins is evidenced by the enormous increase in the number of reports in the literature over the last decade.[1-10] ^{113}Cd NMR spectroscopy has been employed in the study of Ca, Zn, Mg, Cu, Cd, and Hg binding sites in at least 24 different metalloproteins and has provided valuable information on the number of distinct metal binding sites and the nature of the donor groups, particularly when these are all S or all O donors.[8-11] However, inconsistent results sometimes obtained have confounded quantitation of the relationship between ^{113}Cd NMR spectral parameters, such as chemical shift, and the nature of the metal binding sites, particularly when a combination of donor atoms is attached to Cd. As discussed in a review by Armitage and Otvos,[5] a structure–shift correlation would be invaluable for predicting the number, identity, and geometric arrangement of ligands at the metal binding sites of metalloproteins.

This lack of a quantitative structure–shift relationship is due in part to (1) the lability of Cd(II), which in many cases gives rise to rapid-exchange NMR data, and (2) the limited number of suitable model compounds.[5] To overcome the problem of lability, Ellis[4,12] has pioneered the application of ^{113}Cd NMR spectroscopy on models in the solid state, whereas Ackerman[13-15] introduced the use of super-cooled solutions. However, despite the large number of reports on Cd-substituted metalloproteins, there has been disappointingly little published on ^{113}Cd NMR spectra of Cd coordination compounds in solution at ambient temperature.

In previous reports, two of the present authors demonstrated the feasibility of obtaining slow-exchange ^{113}Cd NMR spectra for compounds containing N-donor chelate ligands in Me_2SO-d_6 and D_2O solution.[16,17] Slow-exchange spectra were obtained only for complexes containing the weakly binding oxo-anions NO_3^- or ClO_4^-, and the best results (i.e., better solubility, narrower NMR signals) were obtained in Me_2SO-d_6 solution rather than in D_2O.[16]

In addition, the utility of the ^{113}Cd NMR chemical shift for determination of the number and type of coordinated N atoms for $Cd(L)_n$ complexes was demonstrated. For a series of 14 ligands (L) and 24 $Cd(L)_n$ complexes, including mixed ligand complexes, a formula was derived (eq 1) to calculate shifts which agreed, usually to within 5%, with data obtained in Me_2SO-d_6 solution and referenced to 1.0 M $Cd(NO_3)_2$ in Me_2SO-d_6. Ligands employed included primary, secondary, tertiary, and pyridine N donors:[16]

$$\text{shift} = 75A + 51B + 31C \quad (1)$$

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Table I. ^{113}Cd NMR Data for Complexes of L with $Cd(NO_3)_2 \cdot 4H_2O$ in Me_2SO-d_6[a]

L[b]	[L], M	^{113}Cd NMR δ (% Cd)[c]	δ_{calcd}
$(BzCH_2)_3N$ (1)	0.1	163 (50)	157
	0.2	163 (>95)	157
$(MBzCH_2)_3N$	0.1[d]	163 (50)	157
$((BzCH_2)_2NC_2H_4OCH_2)_2$	0.1	113 (90)	115
$(BzCH_2)_2NC_2H_4N(BzCH_2)(CH_2CH_2OH)$	0.1	189 (50)	188
	0.2	189 (100)	188
$(BzCH_2)_2NH$	0.1	133 (50)	135
	0.2	134 (100)	135
$(BzCH_2)_2NCH_2CH_2OH$	0.1	115 (50)	115
	0.2	115 (95)	115

[a] $[Cd(NO_3)_2 \cdot 4H_2O]$ = 0.20 M; externally referenced to 1.0 M $Cd(NO_3)_2 \cdot 4H_2O$ in Me_2SO-d_6 (δ 0.0). Bz, MBz = 2-substituted benzimidazole and 2-substituted 1-methylbenzimidazole, respectively. [b] Ligand preparations (from top to bottom) are given in ref 29–34, respectively. [c] Values obtained from integrated peak areas. For all data with [L] ≤ 0.2 M, the remaining Cd exists as "free" Cd with chemical shift values of 13–15 ppm. [d] This complex is partially soluble and the actual concentration is somewhat less than indicated.

A, *B*, *C*, = number of primary, secondary, and tertiary (or pyridine) N-donor atoms, respectively. The shift relationship was shown not to hold for complexes in fast exchange.[16] In particular, complexes with unidentate ligands were previously found to be in fast exchange.

As expected, in the present study, complexes formed from another class of chelate ligands containing benzimidazole groups are found to give slow-exchange ^{113}Cd NMR spectra. However, rather surprisingly, on addition of anions (X) such as Cl^-, CN^-, and RS^- to the Cd compound of $(BzCH_2)_3N$[18] (L1; Bz = 2-substituted benzimidazole), new compounds, Cd(L1)X, are formed each of which give a distinct ^{113}Cd NMR signal consistent with slow exchange of X. This novel finding allows for quantitative evaluation of the shift-influence of anions which bind to Cd in model protein environments. Furthermore, a new quantitative donor atom–shift relationship could be derived which reasonably accounts for shifts of some Cd-substituted metalloproteins containing either N and O donors or, in some cases, these donors and one anion donor such as Cl^-, CN^-, or RS^- at the metal binding site.

Experimental Section

Reagents. Me_2SO-d_6 and D_2O employed for NMR studies were from Aldrich. $Cd(NO_3)_2 \cdot 4H_2O$ was from Allied Chemicals or Baker. $Na^{13}CN$ (92.75 atom % ^{13}C) was from KOR. All other reagents were purchased from Aldrich and were used without further purification.

Instrumentation. ^{113}Cd NMR spectral data were obtained with JEOL GX-400 (88.68 MHz) and IBM WP200-SY (44.39 MHz) spectrometers. Data were collected on the JEOL spectrometer under the following conditions: 10-mm NMR sample tubes; ^2H solvent lock; quadrature phase detection; spectral width 80 kHz; preacquisition delay 0.8 ms; pulse width 60°; pulse delay 2.0 s; 16K data points (time domain); 20–60 Hz exponential line broadening. Parameters for data collected on the IBM spectrometer were the same as above except as follows: spectral width 30 kHz; preacquisition delay 50 μs; pulse width 30°; no pulse delay.

Results

Complexes with Weakly Coordinating Oxoanions in Me_2SO-d_6. ^{113}Cd NMR chemical shifts and corresponding signal areas for $Cd(NO_3)_2$ complexes in Me_2SO-d_6 with a new series of ligands containing benzimidazole donors are given in Table I. The observed shifts do not correlate with values calculated via eq 1 by, for example, allowing the shift per benzimidazole to equal the shift per pyridine. Therefore, a new term for benzimidazole donor groups had to be added to eq 1. Analysis of the data in Table I gives a value of 42 ppm for the shift per benzimidazole.

Although the data in Table I were obtained for solutions containing 0.2 M $Cd(NO_3)_2$ and earlier data[16] were obtained with 1.0 M $Cd(NO_3)_2$ solutions, the chemical shift dependence on concentration for $Cd(L)_n$ species appears to be relatively small. For example, the free Cd signal for 0.2 M $Cd(NO_3)_2$ is 11 ppm to lower frequency relative to the shift obtained for 1.0 M $Cd(NO_3)_2$ solutions, and for Cd(*N*,*N*-dmen) (*N*,*N*-dimethylethylenediamine) and Cd(L1) the shift changes by only 8.0 and 2.0 ppm, respectively, on an increase in concentration from 0.2 to 1.0 M. These differences in shift for $Cd(L)_n$ species are less than 8% of the total shifts and have little significance in the interpretations below.

Furthermore, as $NaNO_3$ was added to a solution containing 0.2 M $Cd(NO_3)_2$ and 0.1 M *N*,*N*-dmen, both the free and complexed Cd signals shifted to lower frequency until, at total nitrate = 2.0 M, both signals had chemical shift values only 2 ppm greater than those obtained for a solution initially containing 1.0 M $Cd(NO_3)_2$ and 0.5 M *N*,*N*-dmen. This nitrate dependence provides strong evidence that NO_3^- coordinates, to some extent, to both free Cd(II) and to Cd(*N*,*N*-dmen) and that it rapidly exchanges with solvent nitrate.

Futher evidence for NO_3^- coordination in Me_2SO-d_6 comes from a comparison of ^{113}Cd NMR chemical shifts for compounds made with $Cd(ClO_4)_2$ and $Cd(NO_3)_2$ (Figure 1, bottom line). In this figure, solid symbols represent compounds prepared with $Cd(ClO_4)_2$ and empty symbols are for compounds prepared with $Cd(NO_3)_2$. It can be seen that, for these ligands, shifts are to lower frequency when $Cd(NO_3)_2$ is used compared to $Cd(ClO_4)_2$. However, the difference in the shifts, $\Delta(ClO_4\text{-}NO_3)$, is dependent on L as shown in Figure 1: for L = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (□), *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (▽), *N*,*N*-dimethyldiethylenetriamine (△), and triethylenetetramine (◇), values for $\Delta(ClO_4\text{–}NO_3)$ of 34, 40, 37, and 11 ppm, respectively, were observed. These results indicate that, in Me_2SO-d_6 solution, compounds with ligands containing fewer than 3 N-donor groups have coordinated NO_3^- and that NO_3^- coordinates to a lesser extent to compounds containing more than 3 N donors.

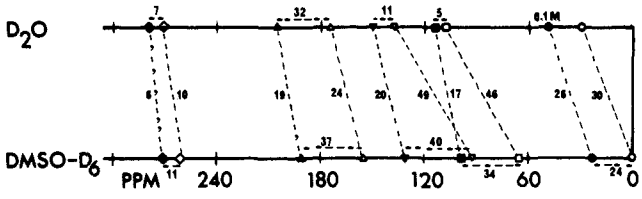

Figure 1. Diagram showing the relative influence of solvent (Me_2SO-d_6 and D_2O) and anion (NO_3^- and ClO_4^-) on the ^{113}Cd NMR chemical shift for free Cd and for Cd(L) compounds. The reference (0.0 ppm) is for a Me_2SO-d_6 solution of 1.0 M $Cd(NO_3)_2$. Solid and empty symbols are for compounds prepared with $Cd(ClO_4)_2$ and $Cd(NO_3)_2$, respectively: L = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (□), *N*,*N*-dimethyldiethylenetriamine (△), triethylenetetramine (◇), and *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (▽).

Table II. ^{113}Cd NMR Chemical Shifts for Complexes of L Ligands with $Cd(NO_3)_2 \cdot 4H_2O$ in Me_2SO-d_6 and with $Cd(ClO_4)_2 \cdot 6H_2O$ in D_2O

L	Δδ, Me_2SO-d_6[a]	Δδ, D_2O[b]
N,*N*,*N*′,*N*′-$(CH_3)_4$en	66	67
N,*N*,*N*′,*N*″,*N*″-$(CH_3)_5$dien	92	102
N,*N*-$(CH_3)_2$dien	155	161
trien	262	229

[a] Reference = 1.0 M $Cd(NO_3)_2 \cdot 4H_2O$ in Me_2SO-d_6. [b] Reference = 0.1 M $Cd(ClO_4)_2 \cdot 6H_2O$ in D_2O.

Complexes with Weakly Coordinating Oxoanions in D_2O. As mentioned above, solubility is often a problem for studies employing D_2O as solvent. Nevertheless, in some cases, ^{113}Cd NMR spectral data can be obtained; see Table II and Figure 1 (top line). Regardless of starting Cd salt, for Cd complexes with all ligands studied, shifts in D_2O were at higher frequency (6 to 46 ppm) than shifts in Me_2SO-d_6 (vide infra).

Complexes with Strongly Coordinating Anions in Me_2SO-d_6. On addition of L1 (0.1 M, Figure 2A; 0.2 M, Figure 2B) to a solution of 0.2 M $Cd(NO_3)_2$, the binding of L1 appears to be

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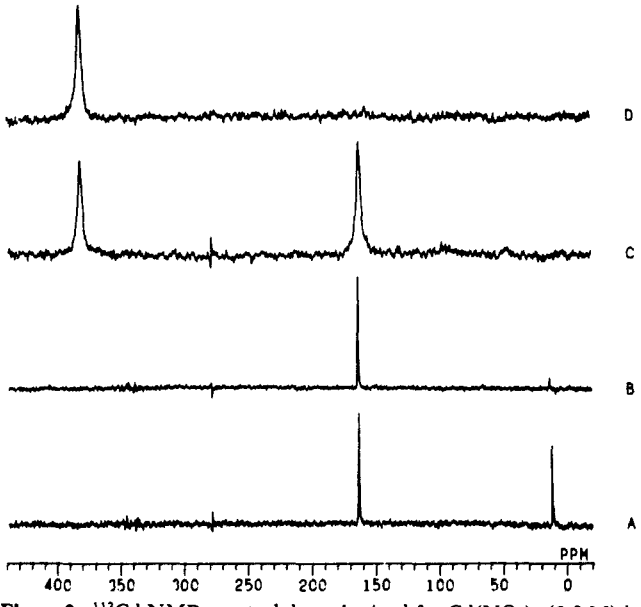

Figure 2. ^{113}Cd NMR spectral data obtained for $Cd(NO_3)_2$ (0.2 M) in Me_2SO-d_6 with added L1 (A, 0.1 M; B, 0.2 M) and NaCl (C, 0.3 M L1, 0.1 M NaCl; D, 0.3 M L1; 0.2 M NaCl).

quantitative with a signal for Cd(L1) at 163 ppm, which increased in area with added L1. Addition of NaCl (0.1 M) resulted in a new signal at 382 ppm, which is of ca. equal area to the signal at 163 ppm (Figure 2C). The high-frequency signal is due to the quantitatively formed Cd(L1)Cl species. Increase in the NaCl concentration to 0.2 M (Figure 2D) resulted in complete formation of the Cd(L1)Cl species, but higher concentrations of NaCl (to 0.3 M, not shown) had no influence on the ^{113}Cd NMR spectrum. Thus, the value of the high-frequency shift induced by Cl^- coordination to Cd(L1) is ca. 220 ppm, and Cd(L1)Cl is in slow exchange with Cd(L1) on the NMR time scale.

Addition of 0.1 M CN^- to the above solution (0.2 M $Cd(NO_3)_2$, 0.2 M L1, and 0.3 M NaCl) produced an extra signal at 403 ppm (Figure 3A). Further addition of CN^- (now as $Na^{13}CN$, 92.75 atom % ^{13}C) to total added CN^- concentrations of 0.2 and 0.3 M (Figure 3, spectra B and C, respectively) resulted in an increase in the area of the signal at 403 ppm with loss of signal intensity at 382 ppm. It is also noteworthy that the 403-ppm signal was substantially broader when ^{13}C-enriched cyanide was added, suggestive of ^{113}Cd–^{13}C coupling. In fact, for a solution containing only 0.1 M $Cd(NO_3)_2$, 0.1 M L1, and 0.05 M $Na^{13}CN$, a sharp doublet ($J(^{113}Cd–^{13}C)$ = 886 Hz) at 397 ppm (Figure 3D) and a singlet of equal area at 163 ppm (not shown) were observed.

The above results indicate that (1) a 1:1 adduct is formed between CN^- and Cd(L1) and (2) CN^- competes with Cl^- for Cd in Cd(L1); however, as shown in Figure 3C, for a solution containing equal amounts of CN^- and Cl^-, the CN^- adduct is preferred only by a factor of ca. 2. It is also apparent from the data in Figure 3C that, for solutions with high CN^- concentrations, polycyano species are obtained. The nature of the complex which gives rise to the multiplet at δ 565 is, at this point, unknown; however, the septet observed at δ 584 is most likely due to formation of $Cd(CN)_6^{4-}$ (an identical signal was observed for a solution containing 0.01 M $Cd(NO_3)_2$ and 0.2 M $^{13}CN^-$).

Addition of the halide ions, Br^- and I^-, gave rise to new signals in the ^{113}Cd NMR spectra for solutions of Cd(L1) in Me_2SO-d_6 (Figure 4). ^{113}Cd chemical shifts for Cd(L1)Br and Cd(L1)I of 374 and 340 ppm, respectively, were obtained. The signals for the Cd(L1) and Cd(L1)X species were broader for X = I^- compared to Br^- and Cl^-, indicative of more rapid chemical exchange for the I^- complex. Fast-exchange NMR data (i.e., a single, broad NMR signal, data not shown; [Cd(L1)] = 0.1 M, [X] = 0.05 M) were obtained for X = F^- (δ 194), N_3^- (δ 180), and SCN^- (δ 190). Fast-exchange NMR results were also obtained when Cl^- was added to Cd(tren) (tren = 2,2′,2″-triaminotriethylamine; δ 276 for Cd(tren)).

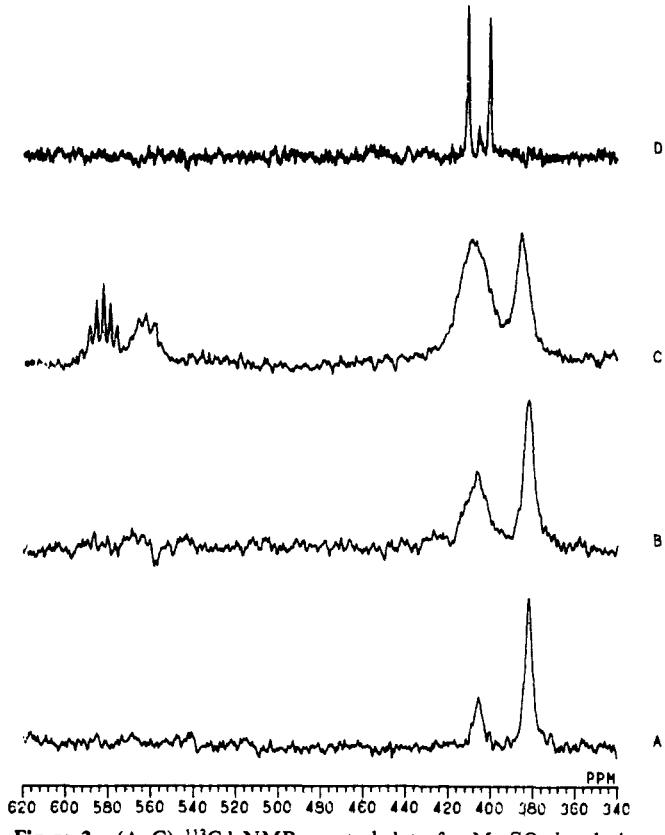

Figure 3. (A–C) ^{113}Cd NMR spectral data for Me_2SO-d_6 solutions containing $Cd(NO_3)_2$ (0.2 M), L1 (0.3 M), NaCl (0.3 M), and CN^- (A, 0.1 M $K^{12}CN$; B, 0.1 M $K^{12}CN$ + 0.1 M $Na^{13}CN$; C, 0.1 M $K^{12}CN$ + 0.2 M $Na^{13}CN$). (D) ^{113}Cd NMR data obtained for a Me_2SO-d_6 solution containing $Cd(NO_3)_2$ (0.1 M), L1 (0.1 M), and $Na^{13}CN$ (0.1 M). In these spectra, signals at δ 382, 403, 584, and 565 are due to Cd(L1)Cl, Cd(L1)CN, $Cd(CN)_6^{4-}$, and an unknown material, respectively.

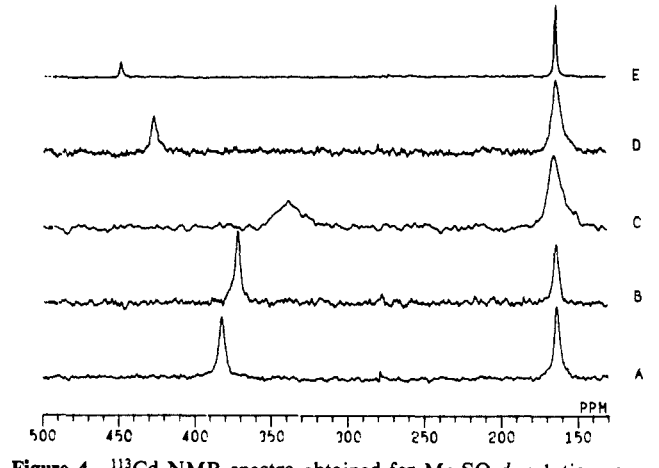

Figure 4. ^{113}Cd NMR spectra obtained for Me_2SO-d_6 solutions containing $Cd(NO_3)_2$ (0.1 M), L1 (0.1 M), and X anions (0.05 M; X = Cl^- (A), Br^- (B), I^- (C), L-cysteine (D), PhS^- (E)). The low-frequency signals at δ 163 and the high-frequency signals are for Cd(L1) and Cd(L1)X species, respectively.

Finally, the sulfide-donor ligands PhSH and L-cysteine were added to Cd(L1) in Me_2SO-d_6. Slow-exchange ^{113}Cd NMR signals at 448 and 431 ppm were observed for Cd(L1)SPh (Figure 4D) and Cd(L1)((*S*)-cysteine) (Figure 4E), respectively. Addition of KOH (to 0.05 M) increased the areas of the high-frequency signals from ca. 25% to 50% of the total peak area without affecting the shifts (data not shown). PhSMe (0.05 M) did not influence the ^{113}Cd NMR spectrum of Cd(L1) (0.1 M), indicating that this neutral sulfur donor does not coordinate. Addition of (S)-methylcysteine (0.05 M) to Cd(L1) (0.1 M) gave a single, broad ^{113}Cd NMR signal at 196 ppm, most likely due to coordination by the amino group. Addition of methionine broadened

but did not shift the Cd(L1) signal.

Discussion

N-Donor Ligand Dependence. The unique relationship between ^{113}Cd NMR chemical shift and the number and type of coordinated N donors[16,17] has been confirmed. From the data listed in Table I, the value for the shift per benzimidazole is 42 ppm; thus, the shift is given by the *modified* eq 2 where *A*, *B*, and *C* have the same significance as in eq 1 and where *D* is the number of coordinated benzimidazoles. The reference (0.0 ppm) is 1.0 M $Cd(NO_3)_2$ in Me_2SO-d_6.

$$\delta_{calcd} = 75A + 51B + 31C + 42D \quad (2)$$

Solvent Dependence. Since studies on metalloproteins are carried out almost exclusively in aqueous media, it is clearly necessary to understand the influence of solvent on the ^{113}Cd NMR chemical shift. To separate the influence of solvent on shift from the influence of anions, mediated by solvent effects, we discuss our results with $Cd(ClO_4)_2$ since ClO_4^- binds weakly to Cd.[19] Analysis of the data in Figure 1 for compounds formed with $Cd(ClO_4)_2$ allows some generalizations to be made. First, the shifts obtained for Me_2SO-d_6 solutions are always to lower frequency relative to the shifts obtained in D_2O. Second, the magnitude of the shift difference for D_2O and Me_2SO-d_6 appears to depend primarily on the accessibility of Cd to solvent. For example, the shift difference for free Cd^{2+} (0.1 M) is 26 ppm. For Cd(L) compounds, the shift difference decreased from 17–24 ppm for L = 2 or 3 N-donor ligands to 10 ppm for the 4 N-donor L = trien (triethylenetetramine), to near zero for $Cd(dien)_2$ (dien = diethylenetriamine) with six coordinated nitrogens.

Anion Dependence. (A) Weakly Coordinating Oxoanions. For Me_2SO-d_6 solutions, all compounds examined which contained less than 4 coordinated N atoms gave $\Delta(ClO_4-NO_3)$ of 34–40 ppm (Figure 1); however, for compounds with 4 or more coordinated N atoms the shift difference is ca. 11 ppm or less (Figure 1). In fact, no difference in shift between the NO_3^- and the ClO_4^- derivatives was observed for Cd(*N*,*N*′-dmen)$_3$ in Me_2SO-d_6.[16] Thus it appears that $Cd(L)_n$ compounds formed from $Cd(NO_3)_2$ in Me_2SO-d_6 with less than 4 coordinated N atoms probably contain rapidly exchanging, coordinated NO_3^-, which causes a low-frequency shift of the ^{113}Cd signal of ~35 ppm.

In D_2O, NO_3^- can influence the ^{113}Cd NMR chemical shift for complexes with less than 4 coordinated N atoms; however, $\Delta(ClO_4-NO_3)$ depends on L in a nonsystematic manner (Figure 1, top line). For complexes with 4 or more coordinated N atoms, NO_3^- has only a minor influence on the shift.

Interestingly, for all species containing less than 4 coordinated N atoms, a parallel relationship exists between shifts observed for $Cd(NO_3)_2$ derived complexes in Me_2SO-d_6 (relative to 1.0 M $Cd(NO_3)_2/Me_2SO$-d_6) and $Cd(ClO_4)_2$ derived complexes in D_2O (relative to 0.1 M $(CdClO_4)_2/D_2O$, see Table II). Perhaps this result should not be surprising since (a) the influence of NO_3^- on shift is roughly the same for all of the compounds with less than 4 N donors in Me_2SO-d_6 and (b) probably none of the compounds in D_2O, including free 0.1 M $Cd(ClO_4)_2$, contain bound ClO_4^-. On the other hand, shifts for compounds with 4 or more N-donor atoms are similar in both solvents ($\Delta\delta$ = 0–10%) regardless of counterion (Figure 1). Note that shifts in Me_2SO-d_6 can be converted to the common reference (0.1 M $Cd(ClO_4)_2$ in D_2O) by adding 50 ppm (see Figure 1).

(B) Strongly Coordinating Anions. In all previous studies, compounds containing coordinated Cl^-, Br^-, and I^- have given rise to broadened, fast-exchange NMR signals confounding identification of anion influence on the ^{113}Cd NMR chemical shift.[16,19,20] In this study, however, slow-exchange ^{113}Cd spectral data were obtained for compounds formed between X^- anions and Cd(L1) (Figures 2–4). High-frequncy shifts induced by X^- binding to Cd(L1) in Me_2SO-d_6 increase in the order I^- (177 ppm) < Br^- (211 ppm) < Cl^- (220 ppm) < CN^- (240 ppm) < [L-cysteine]$^{-1}$ or $^{-2}$ (268 ppm) < PhS^- (283 ppm).

In contrast, earlier mathematical treatments of fast exchange data gave shifts of 92, 70, and 44 ppm for CdX^+ complexes in D_2O with X = Cl^-, Br^-, I^-, respectively.[20] The order agrees with that observed here and is expected for a filled-shell d^{10} ion.[21] The earlier study also indicated that shifts induced by anions are not additive.[20] Indeed, the shift induced by PhS^- binding to Cd(L1) is 283 ppm, whereas shifts for $Cd(SR)_4$ range from 577 to 829 ppm.[22]

Slow-exchange spectra were obtained for CN^-, RS^-, and halide complexes with only Cd(L1). Inspection of models indicates that the remaining coordination position in Cd(L1) is in a "pocket" which could inhibit interactions between the bound anion and other Cd species. Fast-exchange NMR data were obtained for Cd(tren)Cl, which has no pocket, and also for Cd(L1)(SCN) and Cd(L1)(N_3) complexes in which the polyatomic linear anions probably protrude from the Cd(L1) pocket. On the other hand, F^- may bind too weakly to Cd to give slow-exchange spectra.

General Chemical Shift Relationships. The factors which influence the ^{113}Cd NMR chemical shift of Cd complexes are gradually becoming better understood. Some general trends have been known for some time; e.g., deshielding for donor atoms increases in the order O (shielding) < N < S,Se.[4-7,22] Analysis of the ^{113}Cd NMR powder spectra for Cd(TPP) (TPP = tetraphenylporphyrin) and the mono-pyridine adduct, Cd(TPP)py, revealed that py binding causes a 124-ppm shift to lower frequency for $\delta_{\parallel}$ (the unique component of the shielding tensor) and a 112-ppm high-frequency shift for $\delta_{\perp}$ (the in-plane shielding element).[4,12] The isotropic shift ($\delta_i = (\delta_{\parallel} + 2\delta_{\perp})/3$) for the py adduct is 33 ppm to higher frequency from the shift for Cd(TPP),[4] a value which agrees with our solution-derived shift of 31 ppm.

Although the influence of ligands on the shielding tensors for complexes we have studied is not known, the observed trend in chemical shift is followed by a large group of compounds which probably vary widely in structure. Included are Cd complexes with diamines (mono, bis, and tris), triamines (mono and bis), tetramines (both linear and tripodal, including one which formed 6-membered chelate rings) and mixed ligand species with 5-coordinated N atoms. In addition, the types of donors include primary, secondary, tertiary, and aromatic amines. Thus it appears that the primary ^{113}Cd NMR shift determinant for these complexes is the number and type of coordinated N donors. Geometric constraints imposed by ligands probably have a smaller influence on shift, at least in model compounds.

Although eq 2 is successful for Me_2SO, modifications are necessary for analysis of shift data obtained with D_2O solutions. As mentioned above, for fewer than 4 strongly coordinated atoms, shifts for D_2O solutions (reference = 1.0 M $Cd(NO_3)_2/Me_2SO$-d_6) are equal to shifts calculated from eq 2 (δ_{calcd}, reference = 1.0 M $Cd(NO_3)_2/Me_2SO$-d_6). Thus, coincidentally, the correction for the reference cancels the correction for the anion and the solvent (Table II). For compounds with 4 strongly bound ligand atoms, a new correction of 30 ppm is made by subtracting 50 ppm from δ_{calcd} to correct for the reference (see Figure 1) and then by adding 20 ppm to correct for the influence of solvent and anion (see entry for trien, Table II). The new eq 3 now accounts for solvent, reference, anionic ligands (Cl^-, CN^-, RS^-), and number of non-oxygen donors. The value for the RS^- term in eq 3 was obtained from PhS^- data since PhS^- can only be monoanionic whereas L-cysteine may be either mono- or dianionic.

$$\delta'_{calcd} = \delta_{calcd} + 220E + 240F + 285G - 30H \quad (3)$$

E = number of bound Cl^- ligands (0 or 1), *F* = *G* = 0; *F* = number

(19) For ClO_4^- titration results which indicate that ClO_4^- binds weakly to Cd see: Kostelnik, R. J.; Bothner-By, A. A. *J. Magn. Reson.* **1974**, *14*, 141. We thank a referee for pointing out that the correlation between the degree of shielding and the degree of complexation may be difficult to assess as indicated by solid-state CP/MASS studies; see ref 20 and: Mennitt, P. G.; Shatlock, M. P.; Bartuska, V. J.; Maciel, G. E. *J. Phys. Chem.* **1981**, *85*, 2087.

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of bound CN^- ligands (0 or 1), $E = G = 0$; G = number of bound RS^- ligands (0 or 1), $E = F = 0$; $H = 0$ for less than 4 non-O donors and 1 for 4 or more non-O donors.

Relevance to Cd-Substituted Metalloproteins. To relate the ^{113}Cd shifts of eq 3 to those for Cd-substituted metalloproteins, we assume that the shift induced by imidazole is approximately equal to that induced by benzimidazole. Although a complete analysis of all literature data is beyond the scope of this paper, we can nevertheless make some comparisons here and point out discrepancies where they exist and their possible origins. The discussion will be limited to alkaline phosphatase, superoxide dimutase, human carbonic anhydrase C, and Cu proteins.

Alkaline Phosphatase. Alkaline phosphatase (AP) is a dimeric enzyme containing 3 metal binding sites (A, B, C) in each subunit. The C site is comprised exclusively of O-donor ligands, and therefore the ^{113}Cd NMR results for the C site will not be discussed here. The A site has three histidyl (his) ligands and the B site has one his ligand.

For a Cl^--free sample of Cd_2(AP), with Cd at the A site of each subunit, a single ^{113}Cd NMR signal was observed at 117–120 ppm.[2,6] This value corresponds to δ'_{calcd} = 126 ppm. For Cl^--free solutions containing Cd_6(AP)–P (covalently phosphorylated enzyme with Cd(II) occupying all of the available metal binding sites) and Cd_6(AP)·P (noncovalently phosphorylated), chemical shifts of 137 and 133 ppm, respectively, are obtained for the A site. These values agree quite well with the δ'_{calcd} of 126 ppm (Δ, the % error between δ_{obsd} and δ'_{calcd} = 5–8%).

^{113}Cd NMR chemical shift values for Cd in the B site of 54, 64, and 70 ppm were observed[2] for Cd_2(AP), Cd_6(AP)·P, and Cd_6(AP)–P, respectively. Here the agreement with δ'_{calcd} = 42 ppm is fair at best; the range in shifts of exclusively O-donor sites is fairly large (~100 ppm), and we anticipate poorer agreement for sites with fewer than three non-O donors.

Superoxide Dismutase. Superoxide dismutase (SD) is a dimeric enzyme containing single, equivalent Zn binding sites and single, equivalent Cu binding sites in each subunit. Each Zn site has 3 his and 1 COO^- ligands. As for the A site of AP, σ'_{calcd} = 126 ppm. The most recent work on Cd-substituted SD is that of Ellis et al., where chemical shift values for Cd_2(SD) (Cd in the Zn sites) and $Cd_2(Cu^I)_2$(SD) of 311 and 320 ppm, respectively, were obtained.[23] Although different buffers (acetate and sulfate) did not influence the observed chemical shift, $CdCl_2$ was employed, and it is conceivable that Cl^- was still coordinated to Cd. The δ'_{calcd} for Cd containing 3 his and 1 Cl^- as ligands is 316 ppm, which is in excellent agreement with the observed shifts (Δ ~ 1%).

Human Carbonic Anhydrase C. Human carbonic anhydrase C (HC) is a monomeric enzyme containing a single Zn(II). The Zn binding site is comprised of three his ligands; thus δ'_{calcd} is 126 ppm. The observed ^{113}Cd chemical shift for Cd(HC) is highly sensitive to changes in pH, [Cl^-], and [HCO_3^-].[24-26] However, upon addition of $^{13}CN^-$, a chemical shift of 355 ppm ($J(^{113}Cd-^{13}C)$ = 1040 Hz, compare $J(^{113}Cd-^{13}C)$ = 886 Hz for Cd(L1)CN) is obtained.[24] This shift, which is insensitive to changes in pH and [Cl^-], agrees rather well with δ'_{calcd} of 336 ppm for a Cd complex containing 3 his and 1 CN^- as ligands (Δ = 6%). Similarly, the SH^- complex has a shift of 374 ppm[26] which agrees with δ'_{calcd} = 381 ppm (Δ = 2%). It appears that our shift analysis fails under conditions where fast exchange occurs between the Cd(protein) and the Cd(protein)(X^-) species and also where changes in pH markedly influence the ^{113}Cd chemical shift.

Copper Proteins. From X-ray studies the Cu binding sites of azurin and plastocyanin have two histidines, one cysteine, and a neutral thioether or disulfide ligand.[1] The Cu binding site of stellacyanin is probably analogous. ^{113}Cd shifts for Cd-substituted *pseudomonas* azurin, *alcaligenes* azurin, stellacyanin, and *spinacea* plastocyanin of 372, 379, 380, and 432 ppm, respectively, recently have been reported.[1] Since the disulfide and thioether groups are poor ligands for Cd, these may not be coordinated. Except for plastocyanin, the reported shifts agree well with δ'_{calcd} = 369 ppm for 2 his and 1 cysteine ligand (Δ = 17% for plastocyanin and <3% for the others). Indeed, recent heteronuclear multiquantum coherence (HMQC) NMR studies for *spinacea* plastocyanin provide good evidence for the coordination of methionine.[27] If this analysis is correct, then the methionine sulfur induces a further ~50 ppm high-frequency shift. Furthermore, we recently became aware of HMQC studies on azurin and stellacyanin in which coupling is found only to imidazole and cysteine, consistent with our conclusion that no other S donors are strongly coordinated to Cd in these systems.[28]

Conclusion

We are now able to identify slow-exchange ^{113}Cd NMR signals for compounds with coordinated anions, and this finding, along with recent data on proteins,[2] supports the significant influence of anions on shift. In addition, our modified ^{113}Cd NMR shift relationship, which works very well for small molecules, also holds up well for most proteins. For cases where changes in pH significantly influence the ^{113}Cd shift, or where anion-to-Cd(protein) binding is rapid on the NMR time scale, the shift relationship does not hold.

With further study, we may be able to evaluate (a) the reasons for the failure of the shift relationship in the above-mentioned situations, (b) factors influencing ligand exchange rates and mechanisms in small model compounds, and (c) the generality of anion shift values in Cd model compounds with ligands other than L1. Finally, it would be interesting to define situations in which HMQC methods are useful by employing these model compounds. The HMQC method is dependent on $^{113}Cd-^1H$ coupling,[27] and in the absence of such coupling, or in cases where NMR relaxation rates are large, this method is not applicable. Thus, the HMQC and shift techniques are complementary.[35]

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