Contribution from the Department of Molecular Biophysics and Biochemistry and Diagnostic Imaging, Yale University School of Medicine, New Haven, Connecticut 06510, and Division of Physical Chemistry 2, Chemical Center, University of Lund, S-220 07 Lund 7, Sweden

^{113}Cd NMR Studies of Small Dynamically Stable Cd^{2+} Complexes†

ANDREW D. KELLER,§ TORBJORN DRAKENBERG,‡ RICHARD W. BRIGGS,§‖ and IAN M. ARMITAGE*§

Received July 16, 1984

^{113}Cd and ^{13}C NMR spectra have been obtained on several dynamically stable cadmium complexes. In several cases a ^{13}C–^{113}Cd three-bond coupling has been observed and utilized in the assignment of the ^{13}C NMR spectra. The pH dependence of the ^{113}Cd resonance from the cadmium–ethylenediamine-*N,N,N',N'*-tetraphosphonate complex shows that this complex has two acid dissociation constants, pK_a = 7.85 and 8.80, respectively. ^{113}Cd nuclear Overhauser effect, NOE, $=1+\eta$, and spin lattice relaxation time, T_1, were determined on all the complexes and used in the analysis of the contribution to the relaxation mechanism from the dipolar interaction with protons and chemical shift anisotropy. Together, the multinuclear NMR parameters contribute to a detailed understanding of the structure and dynamics of these metal complexes. Furthermore, the ^{113}Cd NMR parameters from these model complexes provide important new data relevant to the interpretation of the ^{113}Cd NMR parameters from biological systems.

Introduction

During the last few years there has been an increasing interest in using ^{113}Cd NMR as a method for studying metalloprotein complexes in which the native diamagnetic metal (e.g., Zn^{2+}, Ca^{2+}) has been replaced by solution.[14-22] isotopically enriched spin $^1/_2$ ^{113}Cd nucleus (for reviews see ref 1–4). Unfortunately, a detailed interpretation of the ^{113}Cd NMR parameters from these biological systems has been impaired as a result of the lack of chemical shift and relaxation data for the ^{113}Cd nucleus in well-defined model systems. These limitations are slowly being resolved, however, and recently there appeared a thorough study of the relaxation mechanisms of the ^{113}Cd nucleus in the Cd–EDTA complex.[5] In addition, an increasing number of papers have appeared where the chemical shifts of ^{113}Cd in dynamically stable cadmium complexes have been reported[6-13] along with several studies dealing with cadmium complexes undergoing fast exchange in solution.[14-22] In the latter studies, the chemical shifts for the various bound species were not obtained directly from the spectra but they could be calculated if all the stability constants were known.[14,15] An alternative solution would be to slow down the exchange sufficiently by decreasing the temperature. An elegant example of this approach using supercooled water emulsions to slow down the exchange in the Cd^{2+}–glycine complexes was first reported by the Ackermans.[16] Finally, unwanted complications due to chemical exchange and/or uncertainties in metal ion coordination from solution complexes can be removed by NMR studies of solid Cd^{2+} complexes by using the combined techniques of ^{113}Cd–^{1}H cross-polarization and magic-angle spinning[23-27] or by using crystals.[28]

In the majority of the Zn^{2+} metalloproteins that have been studied by ^{113}Cd NMR,[1] the metal binding sites are made of nitrogen and oxygen ligands, whereas in the case of the Ca^{2+} binding proteins the sites contain only oxygen ligands.[4] In an effort to more closely model these sites, we have studied the Cd complexes with the ligands shown in Chart I.

Experimental Section

Materials. EDTA and EGTA were commercial products from Sigma. 18-Crown-6 was obtained from Aldrich Chemical Co. The chelator, EDTMP, synthesized from ethylenediamine, formaldehyde, and phosphoric acid by the method of Moedritzer and Irani,[29] was a gift from J. Bock, (University of Pennsylvania). Ligands I–III were gifts from Prof. J.-M. Lehn (Universite Louis Pasteur, Strasbourg, France).

The cadmium complexes were prepared from either $CdCl_2$, freshly precipitated $Cd(OH)_2$, or the ^{113}Cd-enriched chloride, nitrate, acetate, or perchlorate salt. The latter were prepared from ^{113}CdO (96% ^{113}Cd from Oak Ridge Laboratory, Oak Ridge, TN) and the corresponding acid. The 1:1 complexes with EDTMP were formed in H_2O or D_2O from the appropriate cadmium salt titrated with the octaacid EDTMP and either KOH or NaOH with ^{31}P NMR to detect the end point.

Chart I

EDTA = ethylenediaminetetraacetic acid
EGTA = ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid
EDTMP = ethylenediaminetetramethylphosphonic acid

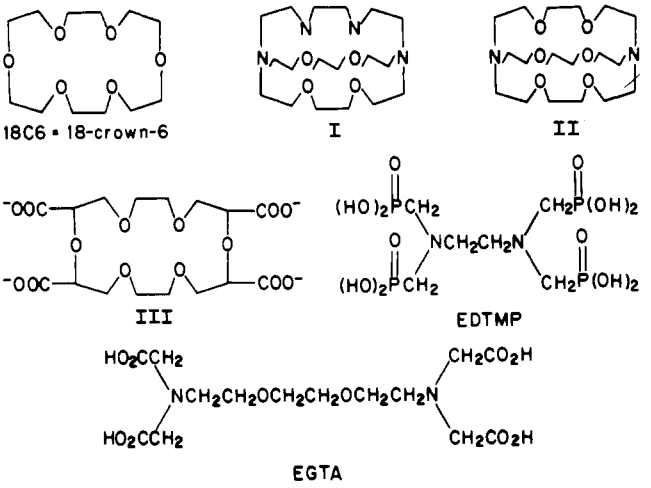

Table I. ^{13}C Relaxation Times (T_1) and Correlation Times (τ_c) for Some of the Cadmium Complexes

ligand	^{13}C T_1,[a] s	$10^{11}\tau_c$, s
EDTA	0.4	6
EGTA	0.3	8
EDTMP	0.15	16
18C6[b]	0.8	3
I	0.8	3

[a] Average of protonated ^{13}C relaxation times. [b] 0.16 M in $CDCl_3$; all other complexes in aqueous solutions.

† This work was supported by National Institutes of Health Grant AM18778.

‡ University of Lund.
§ Yale University School of Medicine.
‖ Present address: Department of Radiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033.

(1) I. M. Armitage and J. D. Otvos in "Biological Magnetic Resonance" Vol. IV, L. J. Berliner and J. Reuben, Eds., Plenum Press, New York, 1982.
(2) P. D. Ellis, *Science* (*Washington, D.C.*), **211**, 1141 (1983).
(3) I. M. Armitage and Y. Boulanger in "NMR of Newly Accessible Nuclei", Vol. 1, P. Laszlo, Ed., Academic Press, New York, 1983.
(4) H. J. Vogel, T. Drakenberg, and S. Forsen, in "NMR of Newly Accessible Nuclei", Vol. I, P. Laszlo, Ed., Academic Press, New York, 1983.
(5) C. F. Jensen, S. Deshmukk, H. J. Jakobsen, R. R. Innes, and P. D. Ellis, *J. Am. Chem. Soc.*, **103**, 3659 (1981).
(6) B. Birgersson, R. E. Carter, and T. Drakenberg, *J. Magn. Reson.*, **28**, 299 (1977).
(7) R. A. Haeberkern, L. Que, Jr., W. O. Gillum, R. H. Holm, C. S. Liu, and R. C. Lord, *Inorg. Chem.*, **15**, 2408 (1976).
(8) H. G. Jakobsen, P. D. Ellis, R. R. Innes, and C. F. Jensen, *J. Am. Chem. Soc.*, **104**, 7442 (1982).

0020-1669/85/1324-1170$01.50/0

Table II. ^{31}P T_1 and NOE Values for Cd(EDTMP) at 2.11 T

compd	solvent	T_1^{obsd},[a] s	NOE (1 + η)	T_1^{DD}, s	T_1^{other}, s
Cd(EDTMP)[b]	H_2O	3.8 ± 0.1	2.01	4.7 ± 0.2	20
^{113}Cd(EDTMP)	H_2O	3.6 ± 0.1	2.07	4.7 ± 0.2	26
^{113}Cd(EDTMP)	D_2O	3.8 ± 0.1	2.02	4.6 ± 0.2	22

[a] Standard deviation from nonlinear least-squares computation. [b] Natural-abundance cadmium.

Spectra. The ^{31}P NMR spectra were recorded on an extensively modified Bruker HFX-90 spectrometer at 36.4 MHz, the ^{13}C spectra at 25.2 or 50.3 MHz on a modified Varian XL-100 or a Bruker CXP-200, respectively, and the ^{113}Cd spectra at 19.96, 22.2, 44.4, 56.5, or 79.9 MHz on a Bruker HFX-90, a Varian XL-100, a Bruker CXP-200, a 6-T NMR spectrometer constructed by H. Lilja,[30] or a Bruker WH-360. All reported ^{113}Cd chemical shifts are referenced to external 0.1 M $Cd(ClO_4)_2$. The ^{113}Cd spin lattice relaxation times, T_1, were measured by using the progressive saturation method of Freeman and Hill.[31] All ^{13}C T_1 determinations employed the inversion recovery method, and both methods were used for the ^{31}P T_1 determinations. Gated ^1H decoupling experiments were performed for all NOE measurements.

Results and Discussion

^{13}C NMR was used primarily as a means for determining the reorientational correlation time of the complexes and, in some cases, the free ligands.[36] The correlation times of the complexes studied in the present work are in the range (3–16 × 10^{-11} s (Table I), and for the open-chain ligands, EDTA, EGTA, and EDTMP, they follow rather closely the variation in molecular weight. The correlation times were calculated from the general equation for T_1^{DD} assuming isotropic motion and a single correlation time. The NOE's for the protonated carbons are all equal, within experimental error, to the theoretical maximum value of 2.99 (1 + η). It might be of interest to note that for ligand I the correlation time is shorter for the complex than for the free ligand (3 × 10^{-11} and 7.2 × 10^{-11} s, respectively). This most likely reflects a more compact structure for the complex, with a nearly spherical shape imposed by the electrostatic metal–ligand interaction.

The carbon-13 NMR spectra in Figure 1 illustrate the stability of the cadmium complex with ligand I. A tentative assignment of the ^{13}C chemical shifts is given on the figure, which is based on substituent effects and the observed ^{113}Cd–^{13}C spin couplings. It is of interest to note that the C_2 symmetry of the free ligand is removed when complexed to cadmium. This can be explained by assuming that the inversion at the two secondary nitrogens has been slowed down to the point where it is slow compared to the nonequivalence in shifts that is introduced by this asymmetry. This would result in the nonequivalence between the halves of each of the oxygen-carrying bridges. Another point of interest in the ^{13}C NMR spectrum of the complex (Figure 1B) is the observation of ^{13}C–^{113}Cd spin coupling. The magnitude of the three-bond ^{13}C–^{113}Cd spin coupling constants measured from Figure 1B are: $^3J_{^{13}C-^{113}Cd}$ = 7 Hz, $^3J_{^{13}C2-^{113}Cd}$ = 4.5 Hz, $^3J_{^{13}C3-^{113}Cd}$ = 12.1 Hz, and $^3J_{^{13}C5-^{113}Cd}$ = 6Hz. While no effort has been made here to rationalize the variation in the ^{13}C–^{113}Cd couplings, for example a possible dependence of the three-bond coupling on the torsional angle, we have used the existence of the couplings to aid in the assignment of the ^{13}C spectrum of the ^{113}Cd–ligand I complex as shown in Figure 1. For example, the observed spin coupling to Cl indicates that the cadmium ion is bound to the secondary nitrogens, and the spin coupling to C5 shows that cadmium is also bound to the tertiary nitrogens.

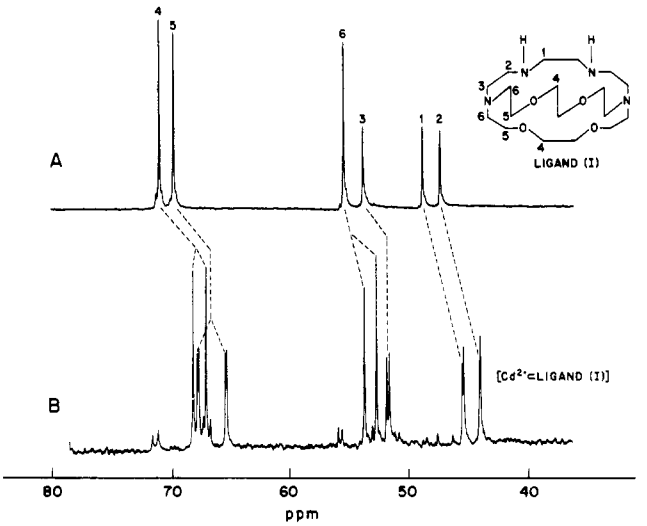

Figure 1. 50-MHz ^{13}C proton-decoupled spectra of (A) ligand I and (B) cadmium complex. The assignments are based in part on substituent parameters and the observed spin couplings.

Table III. ^{113}Cd Chemical Shift, NOE, and T_1 Values

compd	T_1^{obsd}, s	NOE (1 + η)	field, T
^{113}Cd(EDTA) (δ 84, pH 8)	27	−0.5	2.35
	25	0.0	4.7
	29	ND	6.0
^{113}Cd(EGTA) (δ 17, pH 8)	16	−0.22	2.35
	8.8	ND	6.0
^{113}Cd(EDTMP) (δ 104, pH 11)	16	−0.45	2.11
	10	0.0	4.7
	8.5	ND	6.0
^{113}Cd(18C6)[a] (δ 23)	5.7	0.93	2.11
	1.2	0.96	4.7
	0.4		8.47
^{113}Cd(I) (δ 46)	36	−0.5	2.11
	24	0.1	4.7
^{113}Cd(II)[b] (δ −78)			
^{113}Cd(III) (δ_1 −130, δ_2 −8)			

[a] Complex in $CDCl_3$. [b] Complex in Me_2SO.

^{31}P NMR. The ^{31}P NMR spectrum of the free EDTMP ligand shows a singlet at 10 ppm (pH 8.8), which shifts to 15.8 ppm upon

(9) E. C. Alyea, *Inorg. Chim. Acta*, **76**, L239 (1983).
(10) C. C. Bryden and C. N. Reilley, *J. Am. Chem. Soc.*, **104**, 2697 (1982).
(11) G. K. Carson, P. A. W. Dean, and M. J. Stilman, *Inorg. Chim. Acta*, **56**, 59 (1981).
(12) G. K. Carson and P. A. W. Dean, *Inorg. Chim. Acta*, **66**, 37 (1982).
(13) D. D. Domingues, M. M. King, and H. J. C. Yeh, *J. Magn. Reson.*, **32**, 161 (1978).
(14) T. Drakenberg, N.-O. Björk, and R. Portanova, *J. Phys. Chem.*, **82**, 2423 (1978).
(15) J. J. H. Ackerman, T. V. Orr, V. J. Bartuska, and G. E. Maciel, *J. Am. Chem. Soc.*, **101**, 341 (1979).
(16) M. J. B. Ackerman and J. J. H. Ackerman, *J. Phys. Chem.*, **84**, 3151 (1980).
(17) H. J. Jakobsen and P. D. Ellis, *J. Phys. Chem.*, **85**, 3367 (1981).
(18) S. M. Wang and R. K. Gilpin, *Anal. Chem.*, **55**, 493 (1983).
(19) R. J. Kostelnik and A. A. Bothner-By, *J. Magn. Reson.*, **14**, 141 (1971).
(20) R. Colton and D. Dakternieks, *Aust. J. Chem.*, **33**, 1677 (1980).
(21) R. Colton and D. Dakternieks, *Aust. J. Chem.*, **33**, 2405 (1980).
(22) D. Dakternieks, *Aust. J. Chem.*, **35**, 469 (1982).
(23) T. T. P. Cheung, L. E.Worthinton, P. DuBois Murphy, and B. C. Gerstein, *J. Magn. Reson.*, **41**, 158 (1980).
(24) P. DuBois Murphy and B. C. Gerstein, *J. Am. Chem. Soc.*, **103**, 3282 (1981).
(25) P. DuBois Murphy, W. C. Stevens, T. T. P. Gheung, S. Lacelle, B. C. Gerstein, and D. M. Kurtz, Jr., *J. Am. Chem. Soc.*, **103**, 4400 (1981).
(26) H. J. Jakobsen, P. D. Ellis, R. R. Innes, and C. F. Jensen, *J. Am. Chem. Soc.*, **104**, 7442 (1982).
(27) R. F. Rodesiter and E. L. Amma, *J. Chem. Soc., Chem. Commun.*, **1982**, 182.
(28) R. S. Honkonen, F. D. Doty, and P. D. Ellis, *J. Am. Chem. Soc.*, **105**, 4163 (1983).
(29) K. Moedritzer and R. R. Irani, *J. Org. Chem.*, **31**, 1603 (1966).
(30) T. Drakenberg, S. Forsen, and H. Lilja, *J. Magn. Reson.*, **53**, 412 (1983).
(31) R. Freeman, H. D. W. Hill, and R. Kaptein, *J. Magn. Reson.*, **7**, 82 (1972).

complexation of cadmium. Some minor resonances due to impurities in the sample were also observed. In the ^{113}Cd-enriched complex, the resonance at 15.8 ppm appears as a well-resolved doublet (J = 46 Hz). A slight shift to lower field is noted with increasing pH. Excess cadmium and lower pH (<7) both broaden the ^{31}P NMR resonance of Cd(EDTMP).

The ^{31}P T_1 and NOE values determined for Cd(EDTMP) (Table II) clearly show the phosphorus relaxation to be dominated by dipolar interaction with the two methylenic protons. No significant effect on the ^{31}P relaxation due to the anion of the cadmium salt used to prepare the complex or H_2O vs. D_2O are observed. The τ_c value calculated from the T_1^{DD} value, assuming two hydrogens at 2.3 Å and extreme narrowing conditions, is 1.9 $\times 10^{-10}$ s. This value is in reasonable agreement with the value obtained from the ^{13}C relaxation times of this complex (Table I).

^{113}Cd Chemical Shifts. It is now quite well established that the NMR signal from a ^{113}Cd ion bound solely to oxygens is shifted to higher field than when nitrogens are also involved in the metal coordination and to lowest field when sulfur participate in metal ligation.[1]

The Ackermans[16] and Jakobsen and Ellis[17] have shown that the Cd–glycine complexes have ^{113}Cd chemical shifts of ca. 50, 150, and 260 ppm for the 1:1, 1:2, and 1:3 complexes, respectively. This indicates, therefore, that there is an approximately additive deshielding effect of 100 ppm per nitrogen, especially when the chemical shift of free Cd^{2+} at −32 ppm in their medium is taken into account, for all of these complexes that are presumed to be octahedral starting from $Cd(H_2O)_6^{2+}$. These results suggest that one might expect to find the ^{113}Cd(EDTA) signal around 150 ppm and not at ca. 85 ppm as has been observed.[5] Even more surprising is our finding that the ^{113}Cd chemical shifts from the Cd–cryptate complexes with ligands I and II are at 46 and −78 ppm, respectively (Table III), when the observed ^{13}C–^{113}Cd couplings indicate that the Cd^{2+} ion is coordinated to four nitrogens in ligand I and to two nitrogens in ligand II. It is clear from these results that the coordination number, and most likely the geometry of the complex, both must have a strong influence on the ^{113}Cd chemical shifts. The effect from the coordination number has recently been discussed by Ellis[32] on the basis of ^{113}Cd chemical shift data obtained in solid-state ^{113}Cd NMR studies. These data indicate that increasing the coordination number results in increased shielding. In light of this "coordination number effect", we can better understand the ^{113}Cd chemical shift for the cadmium cryptate (I), 46 ppm, as resulting from the coordination of the Cd^{2+} ion to four nitrogens and two to four oxygens. However, the coordination number effect does not explain the difference between the Cd(EDTA) and $Cd(Gly)_2(H_2O)_2$ complexes, which are both octahedral, or nearly so. Even if one takes the effect due to the carboxylate groups into account, there is an unexplained difference of about 50 ppm in the ^{113}Cd shift between these two complexes. This difference might be assumed to be explained by a distortion of the octahedral symmetry in the Cd–EDTA complex; however, there are to our knowledge no data available to validate this assumption.

Despite the fact that the ^{13}C NMR spectrum of an aqueous solution of ligand II and cadmium clearly showed, through an observable ^{13}C–^{113}Cd spin coupling ($^3J_{^{13}C2-^{113}Cd}$ = 10 Hz), that the complex has been formed, we were unable to detect any ^{113}Cd NMR signal for the complex in this solution, presumably as a result of exchange broadening. The exchange must, however, be intramolecular in origin, otherwise the ^{13}C–^{113}Cd coupling would not be observable. In contrast, in Me_2SO solution the ^{113}Cd signal for the Cd–II complex could be observed. Chemical exchange broadening was also observed in the ^{113}Cd resonances from an aqueous solution of the III–Cd^{2+} complex. Even when an excess of ligand III was used, two broad ^{113}Cd resonances of nearly equal areas were observed at −130 and −8 ppm. This indicates that two

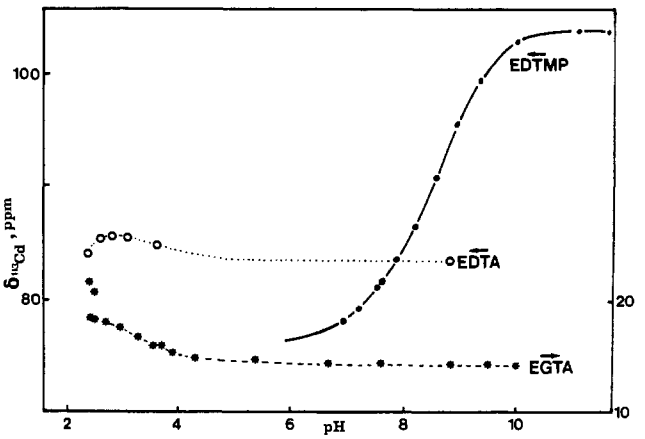

Figure 2. pH dependence of the ^{113}Cd resonance from the complexes Cd(EDTA), Cd(EGTA), and Cd(EDTMP). The chemical shifts for the EDTA and EDTMP complexes are relative to the left axis, and for the EGTA complex, relative to the right axis. The solid curve drawn for Cd(EDTMP) data is calculated with pK_{a1} = 7.85 and pK_{a2} = 8.80 and with shifts of 77, 86, and 104 ppm for $Cd(H_2EDTMP)^{4-}$, $Cd(H_1EDTMP)^{5-}$, and $Cd(EDTMP)^{6-}$, respectively.

different complexes with similar binding constants are formed, one presumably with the Cd^{2+} ion inside the ring and the other with the ion outside the ring. The high-field shift (−130 ppm) is in reasonable agreement with the shift of cadmium ions bound to the calcium sites in calcium-binding proteins,[4,30] and we have therefore assigned this resonance to the $^{113}Cd^{2+}$ ion inside the ligand ring, bound to four carboxylate groups and several of the ether oxygens. This complex could therefore have a coordination number of 8 or more, which would explain the very high-field shift of the ^{113}Cd resonance.

Jensen et al.[5] have performed a very thorough ^{113}Cd NMR study of the Cd–EDTA complex. They found that both the ^{113}Cd chemical shift and relaxation time (T_1) are pH independent in the pH interval 4–11. At higher pH's, the resonance shifts to lower field and the relaxation becomes more efficient. These changes were explained to result from the deprotonation of a coordinated water molecule. We have also studied the pH dependence of the ^{113}Cd chemical shift in the Cd–EDTA, Cd–EGTA, and Cd–EDTMP complexes over the pH interval 2–11. For the Cd–EDTA complex our results agree with those reported by Jensen et al.,[5] and we observed a similar pH dependence for the Cd–EGTA complex (Figure 2). For the Cd–EDTMP complex, on the other hand, the ^{113}Cd chemical shift is strongly pH dependent in the pH interval 6–11. For the Cd–EDTMP complex, on the other hand, the ^{113}Cd chemical shift is strongly pH dependent in the pH interval 6–11. An excellent fit of this experimental data was obtained by assuming two protonation steps with apparent pK_a values of 7.85 and 8.80 (see Figure 2). From these data we can therefore conclude that the pK_a values are 7.85 for $Cd(H_2EDTMP)^{4-}$ and 8.80 for $Cd(HEDTMP)^{5-}$ in reasonable agreement with the data for Cu(EDTMP) and Fe(EDTMP).[33]

^{113}Cd Relaxation. The ^{113}Cd relaxation time will normally have contributions from various mechanisms, and we can write

$$1/T_1^{obsd} = 1/T_1^{DD} + 1/T_1^{CSA} + 1/T_1^{SR} + 1/T_1^{other} \quad (1)$$

where $1/T_1^{DD}$ is the relaxation rate due to the proton cadmium dipole–dipole interaction given by

$$\frac{1}{T_1^{DD}} = \frac{\gamma_I^2\gamma_S^2\hbar^2 I(I+1)}{r^6}\left\{\frac{2}{15}\frac{\tau_c}{1+(\omega_S-\omega_I)^2\tau_c^2} + \frac{2}{5}\frac{\tau_c}{1+\omega_S^2\tau_c^2} + \frac{4}{5}\frac{\tau_c}{1+(\omega_S+\omega_I)^2\tau_c^2}\right\} \quad (2)$$

(32) P. D. Ellis in "The Multinuclear Approach to NMR Spectroscopy", J. B. Lambert and F. G. Riddell, Eds., Reidel Publishing Co., Boston, 1983.

(33) S. Westerback, K. S. Rajan, and A. E. Martell, *J. Am. Chem. Soc.*, **87**, 2568 (1965).

Table IV. Parameters for the ^{113}Cd Nucleus Calculated from the Measured Relaxation Rates

ligand	T_1^{DD}, s	$\Delta\sigma$, ppm	T_1^{SR}, s	T_1^{other},[a] s
EDTA	50	100	100	70
EGTA	31	220	80	
EDTMP	24	155	70	70
18C6		1600		
I	41	240		

[a] T_1^{other} includes contributions from spin rotation and electron-nuclear dipole-dipole and scalar interactions.

$1/T_1^{CSA}$ is the contribution from chemical shift anisotropy given by

$$\frac{1}{T_1^{CSA}} = \frac{2}{15}\omega_{Cd}^2(\Delta\sigma)^2\frac{\tau_c}{1+\omega_{Cd}^2\tau_c^2} \quad (3)$$

and $1/T_1^{SR}$ is the spin rotation contribution given by

$$1/T_1^{SR} = (2IkT/\hbar^2)C_{eff}\tau_j \quad (4)$$

and $1/T_1^{other}$ denotes all other contributing mechanisms such as scalar interactions and electron–nuclear dipole–dipole interactions. τ_j is the angular momentum correlation time, $\tau_c\tau_j = I/6kT$, γ_I and γ_S are the gyromagnetic ratios of proton and cadmium, ω_s and ω_I are the Cd and H resonance frequencies, respectively, in angular units, $\Delta\sigma = |\sigma_\parallel - \sigma_\perp|$ is the anisotropy in the chemical shift, and τ_c is the correlation time.

The dipolar contribution to the ^{113}Cd relaxation has been obtained from the Overhauser effect observed when the proton resonances are saturated, from

$$T_1^{DD} = T_1^{obsd}(\eta_{max}/\eta_{obsd}) \quad (5)$$

where η_{max} and η_{obsd} are the maximal (−2.25) and observed NOE values, respectively.[37] For all of the complexes in the present study, the experimentally measured NOE was less than the maximum values, which implies that at least one of the other relaxation mechanisms is contributing to the relaxation (Tables III and IV).

For the ^{113}Cd–I complex, the dipolar relaxation from protons was used to verify the τ_c value derived from the ^{13}C relaxation. Distances between the cadmium ion and the protons were measured from a molecular model and inserted into eq 2 along with the value for T_1^{DD} obtained from eq 5. The calculated correlation time was about 3.2×10^{-11} s at two field strengths (2.1 and 4.7 T), indistinguishable from the value derived from the ^{13}C relaxation.

The contribution to the relaxation mechanism from chemical shift anisotropy was obtained from measurements of the relaxation at at least two different magnetic fields (ω_A and ω_B) from

$$|\Delta\sigma| = (\Delta(1/T_1^{obsd})/(2/15)[\omega_A^2 - \omega_B^2]\tau_c)^{1/2} \quad (6)$$

In several cases, the observed relaxation could not be explained by these two mechanisms alone and the temperature dependence of the relaxation was determined in order to obtain the contribution from spin rotation since $1/T_1^{SR}$ is proportional to $1/\tau_c$, whereas $1/T_1^{DD}$ and $1/T_1^{CSA}$ are proportional to τ_c. In Table IV we have collected the relevant relaxation parameters. The variation in T_1^{DD} for the three open-chain ligands follows the determined correlation times, and essentially the same ^1H–^{113}Cd internuclear distances can be used to explain this relaxation, 12 protons with $r_{^1H-^{113}Cd}$ = 3.5–4 Å.

More interesting are the large variations in the chemical shift anisotropy, $\Delta\sigma$, that are observed for these complexes (Table IV). A comparison of this parameter between $Cd(EDTA)^{2-}$ and $Cd(EGTA)^{2-}$ leads to the conclusion that the former complex has higher symmetry. This conclusion is in agreement with data on the ^{43}Ca[34] and ^{25}Mg (T. Drakenberg, unpublished results) relaxation in M–EDTA and M–EGTA complexes, which indicate that the EDTA complexes have a higher symmetry resulting in a less efficient relaxation whether the mechanism is quadrupolar as for ^{43}Ca and ^{25}Mg or chemical shift anisotropy as is the case with ^{113}Cd.

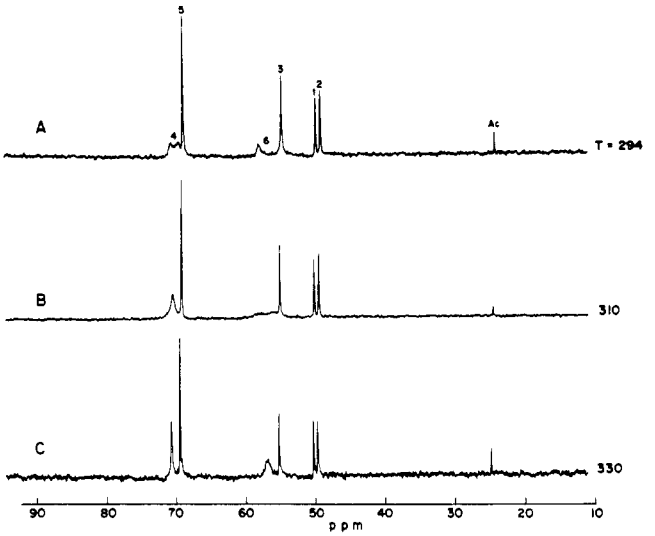

Figure 3. Proton-decoupled 50-MHz ^{13}C NMR spectra of the lead cryptate with ligand I at three different temperatures. The assignments are made in analogy with the cadmium cryptate spectrum of Figure 1.

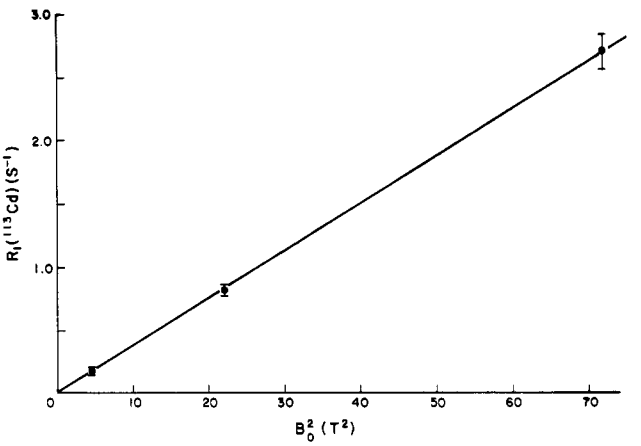

Figure 4. Dependence of the ^{113}Cd relaxation rate, R_1 (s^{-1}), on the field strength for the ^{113}Cd–18C6 complex.

We can rationalize this symmetry difference in the following way. The M–EDTA complexes probably have nearly octahedral symmetry of the ligating atoms, resulting in a small chemical shift anisotropy for the Cd^{2+} ion and small electrical field gradients for the Ca^{2+} and Mg^{2+} ions. In the M–EGTA complexes the ligand has the capability of providing eight coordination sites by using the two ether oxygens, which may result in a less symmetric environment for the metal ion and also a high-field ^{113}Cd chemical shift as compared to the octahedral Cd–EDTA complex.

Particularly striking is the total dominance of the CSA mechanism in the ^{113}Cd relaxation from the Cd–18C6 complex (Figure 3; Table IV). The large CSA determined for the crown ether complex of cadmium may be rationalized in terms of the ligand structure around the metal ion. Crystal structures of monovalent and divalent metal ion complexes of 18-crown-6 show that the oxygen atoms, which bind primarily through electrostatic interactions, are arranged alternately above and below the equatorial plane of the complex, with the axial metal-binding positions free for occupation by anions or solvent molecules. Chloride, the counterion used in the preparation of the 18C6–Cd^{2+} complexes, binds with a fair degree of covalency. Thus, the electrostatic interactions with the equatorially positioned ether oxygens and the more covalent interactions with the axial chloride ions (and/or chlorine of chloroform) could explain the large CSA. Solid-state ^{113}Cd NMR studies of the powder spectra from all

(34) T. Drakenberg, *Acta Chem. Scand., Ser. A*, **A36**, 79 (1982).

of these complexes will be necessary to unequivocally confirm the solution-derived values for the magnitude of the CSA.

Metal Exchange. Addition of a stoichiometric amount of $Pb(OAc)_2$ to a fresh sample of ligand I gave rise to the ^{13}C spectra of the resulting cryptate shown at three temperatures in Figure 4. The ^{13}C NMR spectra of the lead cryptate differs from that of the cadmium cryptate in two ways: (1) There is no observable ^{207}Pb–^{13}C spin coupling (natural-abundance Pb containing 22.6% ^{207}Pb, with $I = {}^1/_2$ was used). (2) There is fast exchange, making the halves of the oxygen bridges equivalent. From the observed broadening of the ^{13}C signals we can estimate the lifetime of the two conformers to be ca. 10 ms at 294 K. These differences between the cadmium and lead cryptates indicate that the lead–nitrogen coordination is weaker than the cadmium–nitrogen coordination.

Mercury forms a complex with ligand I that is much more stable than the cadmium complex, $K_{Hg}/K_{Cd} \approx 10^{13}$. As a result, the former cryptate was formed by direct displacement of cadmium in the presence of an excess of mercury. The kinetics of the exchange is given by

$$Cd^{2+} + L \underset{k_3}{\overset{k_1}{\rightleftharpoons}} CdL \qquad K_1 = k_1/k_3 = 10^{12.7}$$

$$Hg^{2+} + L \underset{k_2}{\overset{k_1}{\rightleftharpoons}} HgL \qquad K_2 = k_1/k_2 = 10^{25}$$

Assuming the same on rate, k_1, for Cd^{2+} and Hg^{2+} because of their similar ionic radii, 0.97 and 1.10 Å, respectively, then the difference in their stability constants must be accounted for by the difference in their off rates, k_3 and k_2. Since $k_2 = (K_1/K_2)k_3 = 10^{-12.3}k_3$, k_2 can be neglected compared to k_3 and therefore once the mercury cryptate is formed, there is essentially no back-reaction. When an excess of mercury is used, we can also assume $[Cd^{2+}] \ll [Hg^{2+}]$, which means that every ligand formed through cadmium dissociation will form the mercury cryptate giving

$$d[CdL]/dt = -k_3[CdL]$$

or

$$[CdL] = [CdL]_{t=0}e^{-k_3t}$$

Either ^{113}Cd or ^{13}C NMR could be used to follow the reaction, and from a ^{13}C spectrum taken 4 days after the reaction was initiated, ca. 57% of the ligand remained in the cadmium cryptate form. This gives a cadmium off rate from the cryptate of $k_3 = 10^{-6}$ s^{-1} at 40 °C, the temperature at which the sample was maintained when not in the spectrometer. According to this, ca. 20 days would be necessary to achieve 95% complexation to mercury.

Conclusion

From the still limited number of model cadmium complexes studied by ^{113}Cd NMR, it would appear that there is as yet no general correlation between the ^{113}Cd chemical shift and the number of coordinating nitrogens. At least three factors affecting the chemical shift have to be considered: (1) nature of the coordinating atoms, with shielding decreasing in the order O > N > S; (2) increased shielding with increasing coordination number; (3) the geometry of the complex.

The small number of model complexes studied thus far suggests that for complexes with a mixture of oxygen and nitrogen ligands a chemical shift anisotropy of 100–200 ppm may be a good estimate for Cd^{2+} ions in an octahedral or close to octahedral geometry. The Cd–18C6 complex with an unusually large value for the chemical shift anisotropy is clearly one example of an exception; however, the conformation of this complex is far from octahedral. We can therefore estimate the CSA contribution to the relaxation of the ^{113}Cd nucleus bound to a metalloprotein to be $1/T_1^{CSA} \leq 0.5$ s^{-1} at a field of 4.7 T and with a correlation time of 10^{-8} s. The contribution from T_1^{DD} cannot be as readily estimated since it is so sensitive to the distance to the nearest proton. However, if one uses proton–cadmium distances similar to those in these model complexes, the result will be that the dipole–dipole contribution will be of the same order of magnitude as the CSA contribution. We will therefore conclude that for small proteins with a molecular weight around 20 000 we should expect to find ^{113}Cd T_1 values on the order of 1 s, whereas for larger proteins it may well increase to several seconds. Qualitatively, these arguments are in agreement with experimental observations for $^{113}Cd^{2+}$ substituted for Zn^{2+} or Ca^{2+} in various metalloproteins.[1-4,35]

Acknowledgment. We thank Professor J.-M. Lehn and Dr. J. Bock for supplying us with some of the ligands. T.D. is grateful to the Swedish Natural Sciences Research Council for financial support. R.W.B. was a NIH postdoctoral fellow (National Research Service Award 1 F32 GM07848-01).

Registry No. ^{113}Cd, 14336-66-4.

(35) P. D. Ellis, P. Y. Yang, and A. R. Palmer, *J. Magn. Reson.*, **52**, 254 (1983).

(36) The value for τ_c was determined from the ^{13}C T_1 and NOE data. For these calculations, the complete general equation for T_1^{DD} was employed. All τ_c values thus evaluated were found to fall within the limits of the extreme narrowing region.

(37) The value of η_{max} of −2.25 is only valid in the extreme narrowing limit that is appropriate for all complexes in this study. The slight (<5%) field dependence of this parameter over the range of frequencies used in this present study is well within the error limits of the experimentally determined T_1 and NOE values.