The Solid and Solution ^{113}Cd N.M.R. Spectra of Six and Seven Co-ordinate Cd^{2+}: Relevance to Ca-substitution Proteins

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The solid and solution ^{113}Cd n.m.r. spectra of a number of benzoyloxy and other carboxylato complexes of Cd^{II} have been measured and chemical shifts in the region of −31 to −60 p.p.m. [more shielded than the $Cd(H_2O)_6(ClO_4)_2$ standard] are correlated with seven co-ordinate oxygen donor species.

^{113}Cd N.m.r. spectroscopy has been shown to be a sensitive probe of metal ion environment in a variety of compounds[1] including metalloproteins and metalloenzymes. A chemical shift range of *ca.* 850 p.p.m. has been observed for the ^{113}Cd nucleus and a chemical shift scale has been established. However, the chemical shift data for ^{113}Cd in the S_2 site of concanavalin A[2] and the EF site of parvalbumin[3,4] have been found to be at *ca.* −100 p.p.m., more shielded than the aqueous 0.1 M $Cd(ClO_4)_2$ standard, and cannot at present be explained from the structure and solution ^{113}Cd n.m.r. spectral data of any model compounds. It has been assumed that the local environment about cadmium in concanavalin A and

parvalbumin is six co-ordinate with oxygen donors. However, examination of the available solution chemical shift data for cadmium complexes containing six oxygen donor atoms shows no examples of chemical shifts in the region of zero to −100 p.p.m.

The ^{113}Cd n.m.r. solution spectra were measured on a highly modified Varian XL-100-15 spectrometer described elsewhere[5] or on a Bruker WP-200 spectrometer, *vide infra*. The ^{113}Cd resonances observed were with natural abundance Cd in solutions of variable concentration ranging from 531 to 88 mM in dimethyl formamide (DMF) and were found to be concentration independent. The line widths were observed to be *ca.* 30 Hz. These values are all shielded with respect to the standard 0.1 M $Cd(ClO_4)_2$ aqueous solution. The resonances are independent of ^{1}H decoupling.

Solid state ^{113}Cd n.m.r. spectra were obtained from *ca.* 0.5 g samples containing the natural abundance ^{113}Cd nuclide on a modified Bruker WP-200 spectrometer at 44.42 MHz (4.7 T) using cross polarization (C.P.) and magic-angle-spinning (M.A.S.) techniques. The contact time was 4 ms, the ^{1}H–90° spin-locking time was 5 ms, and the recycle time was 4 s. Rotor speeds of approximately 4 kHz were employed. A 'solid solution' (finely dispersed mixture) of 'concentration' 0.1 M $Cd(ClO_4)_2$ in Al_2O_3 was used as an external standard.

In order to prepare the desired cadmium compound the appropriate acid (2×10^{-2} mol) and $Cd(NO_3)_2.4H_2O$ (3.2 g) were dissolved in 150 ml of water with gentle warming. Dilute NaOH (0.5 M) was added to bring the pH to approximately 6—6.5. After warming (80 °C) for 45—60 min the solution was cooled to ambient temperature and allowed to evaporate slowly. Crystals appeared in most cases in a few days and were used without recrystallization.

We have measured the DMF and methanol solution ^{113}Cd n.m.r. spectra as well as the solid state (M.A.S.) spectra of a number of benzoyloxy and other carboxylato complexes of Cd^{II} (see Table 1) and determined their crystal structures and thus the metal co-ordination polyhedra. We interpret the data as follows: the seven co-ordinate Cd^{II} species with oxygen donor atoms gives rise to a chemical shift shielded (−30 to −60 p.p.m.) from the six co-ordinate Cd^{II} resonance, as in the $Cd(H_2O)_6^{2+}$ standard with six oxygen neighbours, whether in DMF or MeOH solution or in the solid state. The six co-ordinate Cd^{II} with six oxygen donor species show resonances deshielded in the range 0 to +30 p.p.m. from the standard. This interpretation is clear. A mechanism for the addition of a solution molecule of solvation can be easily envisaged for the six co-ordinate solids upon dissolution, *e.g.*, diaquo(*p*-chlorobenzoyloxy)cadmium(II). However, the substitution of nitrogen donors for oxygen donors makes the situation more complex as indicated by the resonance of the seven co-ordinate tri(pyridine)bis(*o*-hydroxybenzoyloxy)cadmium(II) which is at +61 p.p.m. The 5O, 2N seven co-ordinate Cd^{II} in the monoaquobis(*p*-aminobenzoyloxy)-cadmium(II) complex is an intermediate example. An obvious mechanism also exists for the conversion of the monoaquobis(*p*-aminobenzoyloxy) complex from a polymeric structure with nitrogen donors from adjacent molecules to a seven co-ordinate (7O) species with two oxygen donors provided by the solvent. With more data it may be possible to distinguish in the ^{113}Cd n.m.r. spectrum between the PBP (pentagonal bipyramid) seven co-ordinate Cd^{II} and other seven co-ordinate Cd^{II} co-ordination polyhedra, as well as seven co-ordination with combinations of nitrogen and oxygen donor ligands.

We suggest that ^{113}Cd resonances with oxygen donor ligands in the −30 to −100 p.p.m. region [more shielded than the 0.1 M $Cd(ClO_4)_2$ aqueous solution] represent a higher co-ordinate species than six, that is, at least seven and perhaps more. Therefore, the ^{113}Cd resonances observed in the EF site of parvalbumin and the S_2 site of concanavalin A may be due to higher oxygen co-ordination numbers than six. There may be local conformational changes that occur in these proteins upon replacement of Cd^{2+} for Ca^{2+} (major change); or the metal may simply add an additional water of hydration to its co-ordination sphere without any protein conformational change (minor change). Certainly, replacing Ca^{2+} by Cd^{2+} must bring about some changes (major or minor) from the conformation indicated by the protein structures of parvalbumin[6]† and concanavalin A.[7] In support of this postulate, u.v. difference spectra have been interpreted to indicate that Cd^{2+} induces a slightly different protein conformation in concanavalin A than do Mn^{2+} and Ca^{2+}.[8]

† A recent discussion with Professor Kretsinger indicated that an interpretation of the Ca^{2+} co-ordination in parvalbumin as six is somewhat arbitrary and a higher co-ordination number is consistent with his protein *X*-ray data.

Table 1

Cadmium(II) compound	Co-ordination number [a] and type (oxygen O, nitrogen N)	Solid state (M.A.S.) ^{113}Cd n.m.r. spectrum [c]	Solution ^{113}Cd n.m.r. spectrum [c]
Diaquobis(*o*-hydroxybenzoate)	7O[9] (PBP)	−31	−57 (methanol) −57 (DMF)
Diaquobisacetate	7O[10] (distorted)	−58 −46[14]	−58 (methanol) −46 (DMF)
Bis(*o*,*o*-dimethylbenzoate)	7O [b]	—	−60
Bis(*p*-nitrobenzoate)	6O [b]	+24	−53
Diaquo(succinate)	7O[11] (PBP)	[d]	Insoluble
Bis(benzoate)·xH_2O	6O [b]	+50	−50
Diaquo(*p*-chlorobenzoate)	6O[9] (octahedral)	+29	−51 (methanol) −51 (DMF)
Monoaquobis(*p*-aminobenzoate) dihydrate	7[12] (PBP) 5O, 2N (axial N)	+34	−62 [e]
Hexa-aquaperchlorate	6O[13] (octahedral)	0.0[14]	0 (reference std.)
Tri(pyridine)bis(*o*-hydroxybenzoate)	7[9] (PBP) 4O, 3N	+61	−4.9

[a] From *X*-ray crystallographic structure determinations. [b] Structure determination in progress. [c] Results of this research unless otherwise denoted. [d] Technical complications create temporary problems in observing this resonance. [e] This value is not reliable since crystallization was taking place in the n.m.r. tube during the resonance experiment. PBP = pentagonal bipyramid.

We acknowledge the National Science Foundation Regional Nuclear Magnetic Resonance Facility at the University of South Carolina and the N.I.H. for research support.

Received, 12th August 1981; Com. 992

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