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Structure Determination of Zinc Complexes of Iminodiacetamide Ionophores in Solution and in the Solid State *

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Abstract

It is shown by extensive NMR investigation that hexa-coordinated Zn^{2+}-complexes containing two tridentate iminodiacetamide ligands adopt a solution conformation with two different kinds of Zn–O bonds possessing a center of inversion. In contrast, the complex molecules are chiral in the solid state as determined by CP-MAS ^{13}C NMR spectroscopy.

Introduction

The metal ion Zn^{2+} is a unique member of the essential elements in living organisms. It constitutes the active site of over 300 metalloenzymes but its role is not limited to catalysis and gene expression; Zn^{2+} stabilizes the structure of proteins and nucleic acids, it is involved in transport processes, it is of importance in viral and immune phenomena, etc. [1]. These versatile biological functions of zinc are connected with its peculiar complex-chemical properties: zinc(II) can easily be tetra-, penta- or hexacoordinated without a special preference for the latter geometry [2].

In solid low-molecular-weight complexes zinc is also tetra-coordinated [3–6] in contrast to Co^{2+} and Cd^{2+} but in aqueous solution there is evidence for the existence of hexadentate binding with amino acid derivatives [1].

In the literature numerous bi-, tri- and tetradentate ligands containing sp^3 and sp^2 nitrogen atoms (aliphatic and cyclic primary and secondary amines, amino acids, imidazols, benzimidazols, etc.) were reported to bind zinc salts generally with a tetrahedral or trigonal bipyramidal coordination sphere [1, 2, 4–6]. Of these molecules macrocyclic polyamine-zinc complexes were studied for mimicking the natural carboanhydrase enzyme or used as zinc fluorophores [7]. As part of our ongoing research concerning the development of new electroanalytical metal ion sensors, we aimed at developing a Zn^{2+} selective membrane electrode for biological applications. For this purpose the strongly basic, hydrophilic ligands mentioned previously were unsuitable due to the serious proton interference. We synthesized lipophilic tridentate iminodiacetamide derivatives, and excellent electroanalytical parameters for Zn^{2+} were found when incorporated into PVC membrane electrodes. The alkali and alkaline earth metal ion selectivities of our electrode were rather good ($K_{Zn^{2+}/M^+} = 10^{-3}$–$10^{-4}$) when applied in a blood serum like electrolyte but the detection limit (log DL = −5.5) was unfortunately above that required for *in vivo* monitoring of Zn^{2+} in biological samples under physiological conditions [8]. In order to develop electrodes of higher sensitivity, the structure of the complexes first had to be elucidated since Zn^{2+} complexes of iminodiacetamide derivatives have not been described yet in the literature. From the analytical measurements we indirectly concluded that the complex stoichiometry is probably L_2 ZnX_2. Since this seemed to be quite surprising in the light of the literature results, we prepared complexes with **1a** [8] and **2a** and $Zn(ClO_4)_2$ in ethanol and investigated their stoichiometry by elemental analysis and FAB MS and elucidated their structure in the solution and solid states by NMR methods.

Experimental

General procedures

NMR spectra were recorded in chloroform-*d* at 500/125 MHz on a Bruker Avance DRX-500 spectrometer. Chemical shifts are scaled relative to tetramethylsilane ($\delta = 0$). One- and two-dimensional experiments were performed using microprograms taken from the Bruker software library. HMBC measurements were optimized for 7 Hz long-range couplings. ^{15}N NMR chemical shifts were detected by

* This paper is dedicated to Prof. Dr. András Messmer on the occasion of his 80th birthday.

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^{15}N,^{1}H-HMBC measurements and referenced relative to external nitromethane ($\delta = 0$). CP MAS ^{13}C NMR spectra were recorded under the following conditions: contact time 2 ms (5.1 dB), spinning rate 10 kHz, ^{1}H relaxation delay 5 s. Fast atom bombardment mass spectra (FAB MS) were recorded on a Finnigan MAT 8430 instrument using *m*-nitrobenzyl alcohol as the matrix and Xe as the FAB gas at 9 kV, with an ion source temperature of 25 °C. The CP MAS ^{13}C NMR spectra of benzyl derivatives **2a** and **2b** supported the structural results of the corresponding phenyl derivatives (**1a** and **1b**) but their quality was not good enough to observe signal fine splitting. We expect, however, that the crystal structure properties are analogous.

N-Phenyl-iminodiacetic acid N′,N′-dicyclohexyl-bis-amide **(1a)**. The synthesis was reported previously [8].

N-Benzyl-iminodiacetic acid N′,N′-dicyclohexyl-bis-amide **(2a)**. Benzylamine (0.55 g, 5 mmol), *N,N′*-dicyclohexylbromoacetamide (3.05 g, 10 mmol) and K_2CO_3 (1.8 g, 13 mmol) in 40 mL acetonitrile were stirred under reflux for 6 h. The reaction mixture was filtered while hot and the clear solution was left to stand at room temperature to afford a white solid. Yield: 72%, mp 132–134 °C (MeCN). $C_{35}H_{55}N_3O_2$ (549.85): requires C, 76.46; H, 10.18; N, 7.64; found C, 76.20; H, 10.14; N, 7.60.

Preparation of the complexes **(1b and 2b)**. Compound **1a** and **2a** (0.5 mmol) and $Zn(ClO_4)_2{\cdot}4\,H_2O$ (0.17 g, 0.5 mmol) were boiled in 10 mL EtOH. A white solid was precipitated immediately which was filtered off after chilling, washed with EtOH and dried *in vacuo*. Yield: 75–80%, mp >300 °C (each). **1b** complex: $Zn(ClO_4)_2{\cdot}2\,C_{34}H_{53}N_3O_2$ (1335.92): requires C, 61.13; H, 8.00; N, 6.29; found C, 60.85; H, 8.02; N, 6.19. FAB-MS (positive, isotopic peaks due to ^{37}Cl, ^{66}Zn and ^{68}Zn): *m/z* 1234/1236/1238 $[M–ClO_4]^+$, 698/700/702 $[M–ClO_4–L]^+$. FAB-MS (negative): *m/z* 1432/1434/1436 $[M+ClO_4]^-$, 8 96/898/900 $[M+ClO_4–L]^-$. **2b** complex $Zn(ClO_4)_2{\cdot}2\,C_{35}H_{55}N_3O_2$ (1363.98), requires C, 61.64; H, 8.13; N, 6.16; found C, 61.23; H, 8.10; N, 6.20. FAB-MS (positive, isotopic peaks due to ^{37}Cl, ^{66}Zn and ^{68}Zn): *m/z* 1262/1264/1266 $[M–ClO_4]^+$, 712/714/716 $[M–ClO_4–L]^+$. FAB-MS (negative): *m/z* 1460/1462/1464 $[M+ClO_4]^-$, 910/912/914 $[M+ClO_4–L]^-$.

Results and discussion

The NMR results of the free ligands **1a** and **2a** [R–N{CH_2–CO–N(C_6H_{11})$_2$}$_2$; **1a**: R = Ph, **2a**: R = CH_2Ph] as well as their Zn^{2+} complexes **1b** and **2b** are listed in Table 1. The elemental analyses and the FAB MS spectra revealed the stoichiometry of the complexes with zinc (hexacoordinated) as 2 : 1 (see Figure 1).

The solution NMR spectra of the free ligands show that the two *N*-CH_2 groups are equivalent and only the CH fragments of the cyclohexyl groups gave two different signals due to restricted amide rotation. The effects of complexation can be read from significant signal shifts observed for

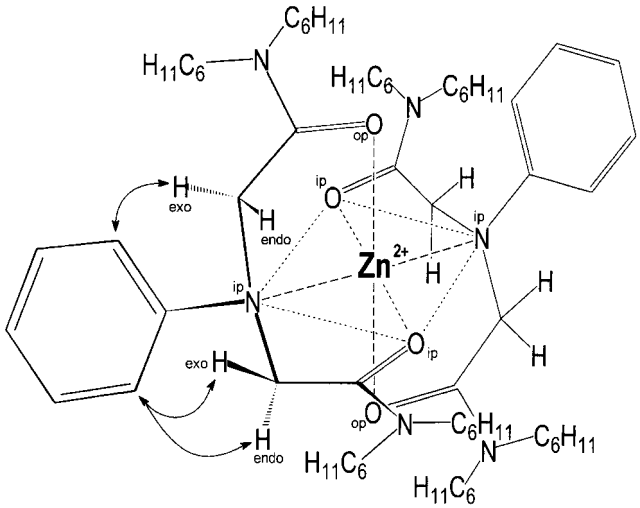

Figure 1. Stereochemical representation of **1b**; double arrows indicate spatial proximities obtained from NOESY spectroscopy. For the explanation of the terms "ip" and "op" see the text.

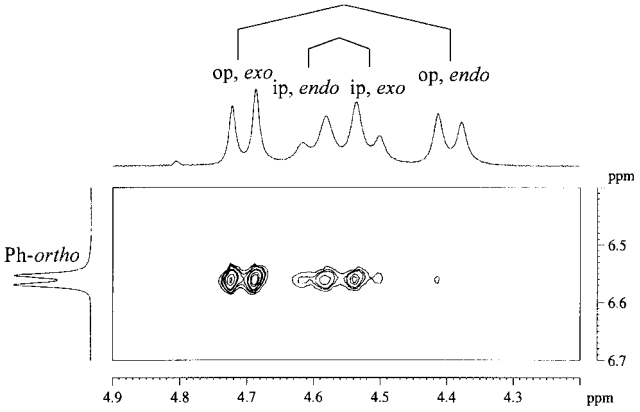

Figure 2. Section of the NOESY spectrum of **1b**. For the interpretation and explanation of the terms "ip" and "op" see the text.

various ^{1}H, ^{13}C and ^{15}N resonances (Table 1). Note, for example, the large deshielding (partial-positive charging) of the *para*-hydrogens ($\Delta\delta = 0.46$) and -carbons ($\Delta\delta = 8.1$) in **1b** transmitted from the Zn^{2+} ions via the nitrogen atom and further across the aromatic system. Protonation or complexation of sp^3 nitrogen atoms is usually accompanied by a small deshielding which is often overcompensated with the steric effect caused by significant changes in bond geometry [9]. In accordance with the above mentioned expectations a 2.7 ppm upfield shift upon complexation of **1** was observed, whereas in the case of compound **2** no change in the ^{15}N chemical shift was found.

Moreover, two well-separated *N*-methylene carbon signals were found at δ = 61.1 and 62.2 in **1b**. Each carbon carries two non-equivalent protons, at δ = 4.70 and 4.39 for the first and δ = 4.52 and 4.59 for the latter. The pairwise attribution and the stereochemical assignment within each of them (*endo* vs. *exo*) could be achieved by EXSY, NOESY and HMQC spectroscopy (Figure 2). Similarly, each of the two signals of the above mentioned cyclohexyl-CH, found in **1a** is split into two: $\delta(^{13}C)$ = 57.2 and 57.5 for one amide branch and 57.7 and 57.8 for the other; pairwise assignments

Table 1. ^{1}H, ^{13}C and ^{15}N chemical shifts of compounds **1a**, **1b**, **2a** and **2b**; in ppm, referenced to tetramethylsilane ($\delta = 0$) for ^{1}H and ^{13}C and nitromethane ($\delta = 0$) for 15N; positive signs refer to deshielding

	1a				**2a**			
	^{1}Ha	$^{13}C^{a}$	$^{13}C^{b}$	^{15}N	^{1}Ha	$^{13}C^{a}$	$^{13}C^{b}$	^{15}N
NCH_2	4.18	54.3	52.0	−323.9	3.46	56.0	55–56	−343.4
Ph-*ipso*	–	148.6	150.5			139.3	138.9	
Ph-*ortho*	6.41	111.6	113.9		7.41	129.4	129.2	
Ph-*meta*	7.13	128.8	128.4		7.28	128.1	∼128[d]	
			127.7					
Ph-*para*	6.65	116.6	116.5		7.23	126.9	∼127[d]	
$(Ph)CH_2$	–	–	–		3.95	59.1	∼60[d]	
NC=O		168.4	168.3			169.7	169.6	
NCH(c.hexyl)	3.48	56.4	55.8	[c]	3.46	56.6	55–56	−233.4
	2.98	56.0	55.8		2.85	55.8	55–56	
	1b				**2b**			
	^{1}Ha	$^{13}C^{a}$	$^{13}C^{b}$	^{15}N	^{1}Ha	$^{13}C^{a}$	$^{13}C^{b}$	^{15}N
NCH_2 op	4.70 *exo*	61.1	61.2	−326.6	4.58 *exo*	61.6	∼62	−343.4
	4.39 *endo*				3.85 *endo*			
NCH_2 ip	4.52 *exo*	62.2	62.7[d]		4.76 *exo*	56.9	57[d]	
	4.59 *endo*				3.80 *endo*			
Ph-*ipso*		148.6	148.6;			132.1	131[d]	
			148.0					
Ph-*ortho*	6.56	117.6	119.3;		7.83	133.0	131[d]	
			116.8					
Ph-*meta*	7.27	129.4	131.1;		7.35	128.3	128[d]	
			128.3					
Ph-*para*	7.11	124.7	123.1		7.39	129.2	128[d]	
$(Ph)CH_2$	–	–	–		3.76; 4.04	61.9		
NC=O op		170.6	171.8			170.0	169[d]	
NC=O ip		171.4				170.9	169[d]	
NCH(c.hexyl) op	3.05	57.2	55.5	[c]	2.90	57.1	57[d]	[c]
op	3.21	57.5	57.8[d]		3.11	57.2	57[d]	
ip	3.96	57.7	58.7[d]		3.81	57.4	57[d]	
ip	3.85	57.8	59.8		3.72	57.5	57[d]	

[a] In chloroform-*d* solution.
[b] CP-MAS data.
[c] Not identified.
[d] Unresolved.

followed from the EXSY cross-peaks. The corresponding ^{1}H signals are $\delta(^{1}H) = 3.05$, 3.21, 3.96 and 3.85, respectively. This leads to the conclusion that molecule **1b** does not contain symmetry planes. (The corresponding data of **2b** are $\delta(^{13}C) = 57.1$ and 57.2, 57.4, 57.4 and $\delta(^{1}H) = 2.90$, 3.11, 3.81 and 3.72, respectively.) Thus, the coordination sphere around the zinc ion is not symmetrical; i.e., there are two different Zn–O bond lengths for each ligand molecule. On the other hand, the two ligand molecules in the complex are equivalent; otherwise, four signals for each *N*-CH_2 instead of two and eight CH instead of four would be observed. Thus, it turns out that the complexes possess a center of inversion (S_2) in solution. As shown in Figure 1 by dotted lines, we arbitrarily assigned a plane which includes the shorter Zn–O bond lengths ("in-plane", ip) recognized by their larger carbonyl ^{13}C chemical shifts (**1b**: $\delta = 171.4$; **2b**: $\delta = 170.9$) and, consequently, a stronger deshielding. The other carbonyls with the longer Zn–O bonds and smaller ^{13}C chemical shifts (**1b**: $\delta = 170.6$; **2b**: $\delta = 170.0$) is designated "out-of-plane" (op).

The solid-state ^{13}C NMR spectra are even more complicated although there are no major chemical shift differences for corresponding carbons in the complex as compared to the respective free ligand (Figure 3). E.g., a small but significant signal splitting for the *meta*-carbons of **1a** was found proving that the phenyl groups are no longer in symmetrical positions in the solid state (although no corresponding signal doubling at further atoms were observed although some signal broadenings were observable).

In contrast, signal splitting is much more pronounced in the zinc complex **1b**. Even for the cyclohexyl-CH carbons more than four signals (eight is assumed, Figure 3b) can be identified, and NCH_2 gives more than two signals. Therefore, we conclude that in the solid state the local symmetry around the zinc ion (formerly the center of inversion) is reduced.

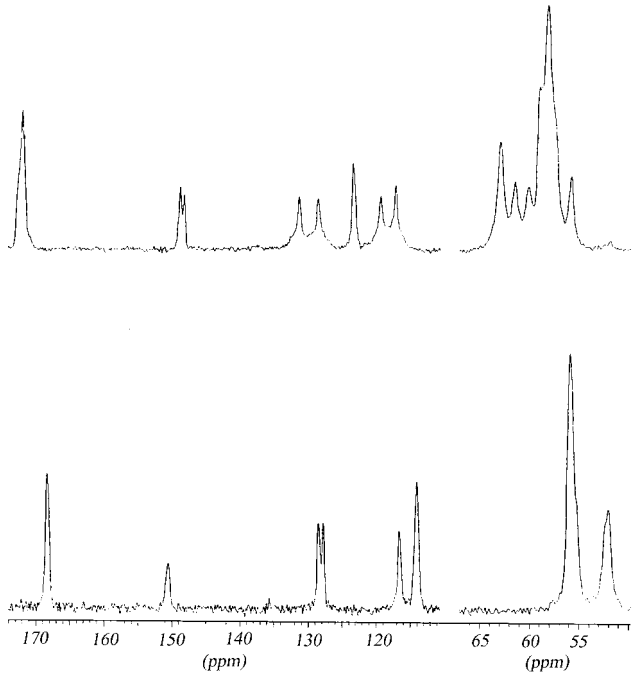

Figure 3. Sections of the CP-MAS ^{13}C NMR spectra of **1a** (lower) and **1b** (upper); carbonyl and aromatic region (δ = 174–110 ppm), *N*-CH_2 and cyclohexyl-CH region (δ = 67–50 ppm).

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