The Complex Formation of Cadmium(II) With Guanosine

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Abstract

The interaction of Cd(II) with guanosine (LH) is examined by means of potentiometric titration and $^{1}H/^{13}C$ NMR. Cadmium(II) forms 1:1 complexes of type CdL^{+} and $CdLH^{2+}$; the pK values for these species are −3.13 and −1.58, respectively. The CdL^{+} species occurs above pH = 6.8, while $CdLH^{2+}$ exists over the whole pH range investigated. The cadmium(II) complex is more stable than the analogous one of zinc(II). Spectroscopic data suggest simultaneous binding of Cd^{2+} to N_7 and O of the guanosine base.

Introduction

As result of experimental studies on biological activities of cadmium conducted by many investigators, it was found that Cd(II) causes damage in living organisms [1–5]. Cadmium has been reported also as carcinogenic in animals, as it was found that cadmium chloride at 10^{-3} M concentration can induce changes suggestive of base mispairing between poly(I) and poly(C, U) [5].

Although interactions between cadmium and biological ligands have been studied and a variety of methods has been applied [6–10], there are several aspects to be investigated yet. It seemed therefore of primary interest to determine types and stability of complex species formed by Cd(II) and nucleic bases to obtain more details for possible underlying eventual metal induced mechanisms for nucleic base mispairing reactions [11].

For our experiments we selected the interaction of Cd(II) with guanosine, serving as a model for DNA base interactions with this ion.

Experimental

Materials

$Ca(NO_3)_2 \cdot 4H_2O$, $Cd(Cl)_2$ (analytical grade) and KNO_3 (analytical grade) were obtained from Merck and Fluka, respectively. Guanosine was obtained from Fluka AG.

All aqueous solutions were prepared using deionized water; the ionic strength was kept constant at 1.0 M KNO_3 in the potentiometric measurements. The concentration of cadmium solution and the ligand were 0.05 M and 0.01 M, respectively. Acid and base, HNO_3 and NaOH, were Merck Titrisol products.

For NMR experiments DMSO-d_6 (Fluka) was used after drying over a molecular sieve.

Apparatus

For pH measurements a Schott pH meter CG 803 and a standard glass electrode were used. Potentiometric measurements were carried out in a thermostatized cell.

Potentiometric Titrations

Potentiometric titrations were carried out at 25 °C. For the study of protonation and deprotonation of guanosine, three titrations curves with 305 experimental points were recorded in which the ligand concentration was 0.00025 M.

For the Cd(II) complex formation with guanosine, titrations were carried out within metal/ligand ratios from 7:1 to 1:5. The concentrations of ligand and metal varied from 0.0001–0.0005 M and 0.0001–0.0015 M, respectively and 480 experimental points from eight titrations were used for evaluation.

Calculation of Equilibria Constants

For the calculation of the stability constants from the potentiometric data the SUPERQUAD program [12] was used. The following equilibria have been considered for complex formation

$$LH + H^{+} \rightleftharpoons LH_2^{+} \quad (1)$$

$$LH_2^{+} + H^{+} \rightleftharpoons LH_3^{2+} \quad (2)$$

$$LH \rightleftharpoons L^{-} + H^{+} \quad (3)$$

$$Cd^{2+} + LH \rightleftharpoons CdLH^{2+} \quad (4)$$

$$Cd^{2+} + L^{-} \rightleftharpoons CdL^{+} \quad (5)$$

$$Cd^{2+} + 2L^{-} \rightleftharpoons CdL_2 \quad (6)$$

$$Cd^{2+} + 2LH \rightleftharpoons Cd(LH)_2^{2+} \quad (7)$$

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$$Cd^{2+} + LH_2^+ \rightleftharpoons CdLH_2^{3+} \quad (8)$$

$$Cd^{2+} + H_2O \rightleftharpoons CdOH^+ + H^+ \quad (9)$$

$$CdOH^+ + LH \rightleftharpoons Cd(OH)LH^+ \quad (10)$$

All calculations were carried out on the CDC cyber 840 computer of the University of Innsbruck.

Results and Discussion

Potentiometric Titration

Cadmium(II) forms 1:1 complexes of type CdL^+ and $CdLH^{2+}$. If LH denotes the neutral form of the ligand, the following reactions were shown to describe the system satisfactorily within the whole pH range from 1–9.5:

$$LH \rightleftharpoons L^- + H^+ \qquad K_1$$

$$LH + H^+ \rightleftharpoons LH_2^+ \qquad K_2$$

$$Cd^{2+} + L^- \rightleftharpoons CdL^+ \qquad K_3$$

$$Cd^{2+} + LH \rightleftharpoons CdLH^{2+} \qquad K_4$$

$$Cd^{2+} + H_2O \rightleftharpoons CdOH^+ + H^+ \qquad K_5$$

The stability constants for these reactions are reported in Table 1.

The values for the protonation constants agree well with those reported by other investigators. Guanosine experiences protonation in the pH range 1–4 and deprotonation above pH = 7. Protonation of guanosine is usually assumed to occur at the N_7 atom [17].

Under our experimental conditions, which can be considered closely related to those of biological systems, the dominant species formed are the cadmium complexes $CdLH^{2+}$ and CdL^+. The CdL^+ species occurs above pH = 6.8 while $CdLH^{2+}$ exists over the whole pH range investigated.

The stability constants of Cd(II) complexes are relatively small. However, when comparing the value for $CdLH^{2+}$ species to the corresponding Zn(II) complex of guanosine (pK $ZnLH^{2+}$ = 1.20 [13]), it is obvious that the cadmium complex is more stable than that of zinc. In the case of Zn(II), the species ML^+ has not been detected up to pH = 7.

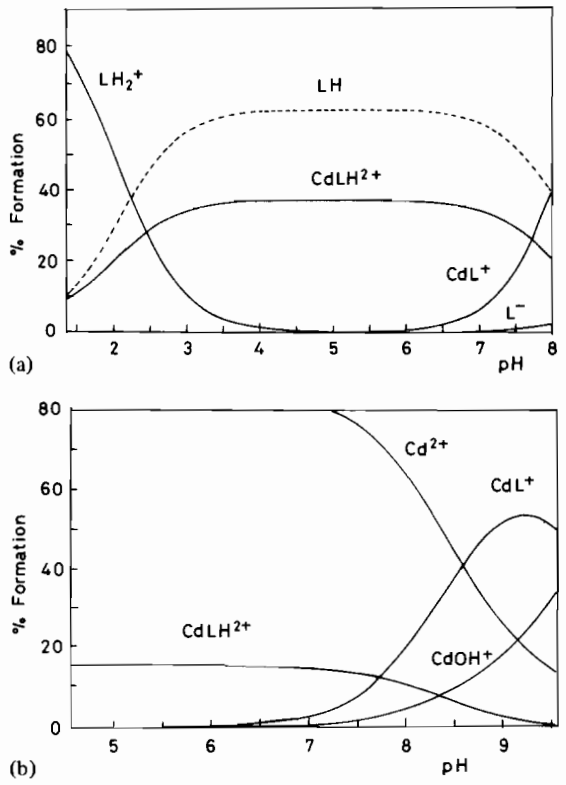

Fig. 1. Species distribution as a function of pH for Cd(II)–guanosine. (a) Concentration is given in percent of total ligand concentration (C_L = 0.000500 M). (b) Concentration is given in percent of total metal concentration (C_M = 0.00100 M).

Figure 1a and b illustrate the species distribution as a function of pH for the Cd(II)–guanosine system. Around pH = 7, which is the relevant pH range for biological systems, 20–30% of Cd(II) and guanosine are present in the form of complexes. At pH = 7.7, species $CdLH^{2+}$ and CdL^+ exist in almost equal amounts, indicating easy conversion of these cadmium–nucleic base adducts upon slight changes of pH. These findings provide evidence that Cd(II) can interact in a significant and versatile way with nucleic bases.

Due to the small values of the stability constants of the Cd(II) complexes, the corresponding species distribution will be sensitive to small changes in

TABLE 1. Stability constants from potentiometric investigations[a]

	pK_1	pK_2	pK_3	pK_4	pK_5
This work	9.29 ± 0.01	−2.27 ± 0.03	−3.13 ± 0.03	−1.58 ± 0.04	−9.60
Literature	8.93 [13][b]	−1.90 [13][b]			
	9.31 [14]	−2.23 [16]			
	9.24 [15]	−2.20 [15]			

[a]For definition, see text. [b]At 37 °C.

the Cd(II) concentration present. The magnitude of the constants provides good evidence with respect to polynucleotide related activity of Zn(II) and Mg(II) [18, 19] that even small amounts of Cd(II) can induce base mispairing in DNA/RNA as already observed between poly(I) and poly(C, U), and therefore cause damage in living organism through this mechanism.

It seems highly possible that Cd(II) can directly bind to the bases of DNA/RNA at elevated concentrations, in the same way as the biologically essential Zn(II) ions. Once this binding occurs, Cd(II) will be removed less easily than Zn(II).

NMR Experiments

For recording of NMR spectra, a 0.1 M solution of guanosine was prepared in anhydrous DMSO-d_6. ^1H and ^{13}C spectra were recorded for this solution and one containing in addition anhydrous cadmium chloride (0.5 M), at room temperature on a BRUKER AM 300 NMR spectrometer.

Remarkable shifts of ^{13}C and ^1H signals are observed in the presence of cadmium chloride. The most significant changes are summarized in Table 2. These changes also give strong indications towards the most probable structure of the cadmium complex formed. Whereas proton shifts are assumed to be mostly due to interactions with chloride anions, in both guanine and ribose parts of guanosine, ^{13}C shifts seem to be mainly influenced by the cation. The most characteristic changes occur at C_8, C_6, C_2 and C_5 of the guanine part. The simultaneous downfield shifts of C_8 and C_6 could be understood by assuming coordination of the cation to N_7 and O (C=O), forming a chelate structure, as such a coordination would induce charge transfer to the coordination sites, and hence a more positive charge at adjacent carbon atoms. For C_5, this would mean opposite effects arising from C_6 (increase of density) and N_7 (induction of a positive charge). Apparently the C_6 effect dominates, and this also explains the large shift at C_8 (electron density for N_7 is accumulated from one neighbouring atom only). If the cation were bound to N_7 alone, density could have been transferred easily from C_8 and C_5 as well, and a similar shift should have been observed for both carbon signals. In the literature, coordination to N_7 is usually mentioned [20], based on X-ray structural data. The formation of a chelate complex could therefore be a characteristic of the solution and the larger cation.

TABLE 2. ^{13}C and ^1H NMR shifts (ppm) induced in guanosine signals by cadmium chloride in DMSO-d_6 and electron density changes (electrons) induced by zinc(II) upon binding to N_7... O(CO) of guanine, according to *ab initio* calculations

Atom	Shift (ppm)	Density change (e)
C_8	−0.449	−0.457
C_6	−0.200	−0.110
C_2	−0.199	−0.035
C_5	+0.192	+0.117
C_4	−0.057	−0.071
H(NH)	−0.080	−0.077
H(NH2)	−0.027	−0.062
H(CH-Im)	−0.022	−0.091
H(OH-ribose)	−0.020/−0.030	

Negative sign denotes downfield shifts and loss of electron density, respectively.

In order to examine the validity of our assumptions concerning charge transfer within the guanine part of the molecule upon metal coordination, a comparison with *ab initio* calculated atomic population changes upon binding of zinc ion to guanine [21] has been made. In these calculations, coordination to N_3, N_7 and N_7... O(CO) had been examined. Only the density changes obtained for the latter configuration are in agreement with our observed shifts, and they are given in Table 2 for comparison. Both the upfield shift of C_5 and the larger extent of effects on C_8 are well reflected. We therefore assume that binding of the cadmium ion in the form of a N_7... O(CO) chelate structure is most likely to occur in aqueous solution. According to the guanine–zinc complex calculations [21], this position is also energetically favoured for the isolated system. The higher stability of the Cd(II) complex compared to that of Zn(II) is a further indication for a chelate formation, which should be more easily realized with the larger ion.

$CdLH^{2+}$ is the dominant species over most of the pH range investigated, and NMR experiments as well as calculations refer to this form of the ligand. It is not very likely, however, that deprotonation, leading to the species CdL^+ (dominating only beyond pH 8), will lead to drastic changes in complex structure. Low solubility of guanosine and exchange processes in aqueous solution have so far prevented the recording of reliable NMR spectra for solutions, where this species would be dominant.

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