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Zinc(II) and Cobalt(II) Induced Amide Deprotonation in Bis((N-2-acetamido)iminodiacetato) Chelates

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Since the discovery of metal ion induced peptide (amide) linkage deprotonation reactions in 1956 [1], many studies have led to the conclusion that of the first row divalent transition metal ions only Cu(II), Ni(II), and Co(II) are capable of inducing amide deprotonation reactions. In our continuing studies of unusual metal ion deprotonation reactions [2], we now report the first case of amide deprotonation induced by Zn(II).

The potentiometric formation curves (not shown) of 2:1 N-2-acetamidoiminodiacetic acid (H_2ADA) to Zn(II) and Co(II) show a high pH buffer zone above a = 2.0 moles of base per mole of ligand. The data can be analyzed [3] in terms of stepwise deprotonation of the amide protons in $[M(ADA)_2^{2-}]$ (eqns. 1 and 2).

$$M(ADA)_2^{2-} \xrightleftharpoons{K_{1a}} M(H_{-1}ADA(ADA)^{3-} + H^+ \quad (1)$$

$$M(H_{-1}ADA)(ADA)^{3-} \xrightleftharpoons{K_{2a}} M(H_{-1}ADA)_2^{4-} + H^+ \quad (2)$$

Values for the protonation constants of H_2ADA and Zn^{2+} and Co^{2+} complex formation constants are given in Table I. The K_1 and K_2 values are similar to those obtained by Schwarzenbach [4]; however, no amide deprotonation was reported [4], probably due to the fact that such reactions were unknown at that time.

In order to confirm amide deprotonation in $[M(ADA)_2^{2-}]$ complexes, infrared, visible, conductivity, and NMR studies were carried out. The infrared data [5], did not unfortunately unambiguously confirm amide deprotonation. Visible data [6] clearly indicate that $[Co(ADA)_2^{2-}]$ undergoes amide deprotonation reactions. From **a** = 2.0 to 3.0, a series of spectral shifts (color change, pink to violet to blue) occurs, which cannot be due to hydrolysis of the amide groups to form bis(nitrilotriacetato)-Co(II), which is pink in aqueous solution. Similar pink to blue [7] (violet) [8] color changes have been reported to accompany amide deprotonation in bis-(glycylglycinato)Co(II) [7] and diglycylethylenediaminetetraacetato Co(II) [8]. Martin [7] has attributed the pink to blue color change as signifying a partial high spin (pink) to low spin (blue) conversion upon amide deprotonation. Ionized amide groups have large ligand field effects as shown by visible spectra of Cu(II) chelates [9] and by the conversions of high spin (octahedral) to diamagnetic (square planar) complexes in Ni(II) chelates of small peptides [10]. It is interesting to note that the amide deprotonation reactions in $[Co(ADA)_2^{2-}]$ are stepwise (see K_{1a} and K_{2a} values in Table I and no isobestic point was found between **a** = 2.0 and 3.0, and not cooperative as reported [7] for bisglycylglycinatoCo(II) and other bispeptide Co(II) chelates.

NMR data [11] shown in Fig. 1 provides spectral evidence of amide deprotonation in $[Zn(ADA)_2^{2-}]$. The free ligand ($ADAH^-$) spectrum 1, has two absorbances (4.12 and 3.77 ppm) with relative integrations of 1:2, assigned to methylene protons of amidomethyl and carboxylmethyl groups, respectively. Deprotonation of $HADA^-$ to yield ADA^{2-} (spectrum 2) also exhibits two resonances 3.35 and 3.26 ppm, indicating more shielding due to the loss of the zwitterionic proton of $HADA^-$. Spectrum 3 of a 1:1 mixture of [Zn(ADA)] and $HADA^-$ exhibits two fairly broad absorbances at 4.00 and 3.76 ppm (relative integration 1:2) assigned to methylene resonances of amidomethyl and carboxylmethyl groups, respectively. The broadness of the peak and lack of bands at 4.12 and 3.77 ppm ($HADA^-$) are probably due to the following equilibrium (eqn. 3). The fourth spectrum of $[Zn(ADA)_2^{2-}]$ consists of

$$Zn(ADA_1) + HADA_2^- \rightleftharpoons Zn(ADA_2) + HADA_1^- \quad (3)$$

TABLE I. Formation Constants of M–ADA Complexes[a].

	log K_1	log K_2	log K_{1a}	log K_{2a}
H^+	6.67[b,c]	2.31		
Co^{2+}	6.72[b]	2.62 ± 0.01	−10.03 ± 0.02	−11.34 ± 0.02
Zn^{2+}	7.10[b]	2.12 ± 0.02	−9.49 ± 0.01	−10.56 ± 0.01

[a] At 25.0 °C and 0.1 *M* (KNO_3). [b] R. Nakon, *Analyt. Biochem.*, *95*, 527 (1979). [c] log K_3 = 1.59 ± 0.03.

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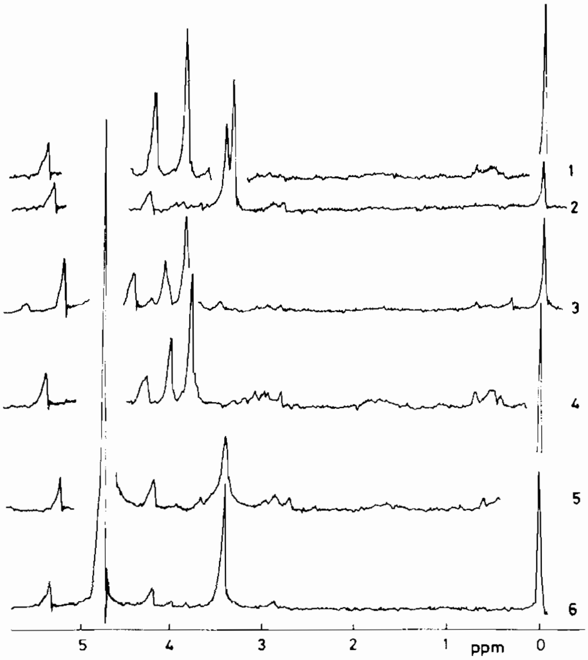


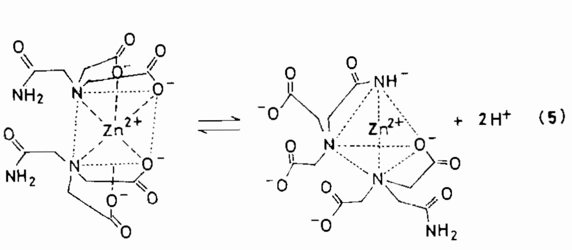
Fig. 1. NMR spectra of $ADAH_2$ and 2:1 $ADAH_2$ to Zn^{2+} solutions (D_2O) at various a values 1) ADA^-, 2) ADA^{2-}, 3) 1:1 mixture [Zn(ADA)] and $ADAH^-$, 4) $[Zn(ADA)_2^{2-}]$, 5) $[Zn(H_{-1}ADA)(ADA)^{3-}]$, and 6) $[Zn(H_{-1}ADA)_2^{4-}]$. Peak at 4.80 ppm is due to HDO, and DSS (0.0 ppm to TMS) was used as standard.

two resonances at 3.90 and 3.67 ppm corresponding to the methylenic protons of the amidomethyl and carboxymethyl moieties, respectively. The fifth and sixth spectra are of $[Zn(H_{-1}ADA)(ADA)^{3-}]$ and $[Zn(H_{-1}ADA)_2^{4-}]$, respectively, and contain only one peak at ~3.33 ppm, corresponding to absorption by the methylene protons both of the amidomethyl and carboxymethyl moieties. In order to show that the single absorbances in spectra 5 and 6 were not due to the hydrolysis of $[Zn(ADA)_2^{2-}]$ to $[Zn(NTA)_2^{4-}]$, a 0.18 *M* solution of $[Zn(H_{-1}ADA)_2^{2-}]$ was allowed to remain at room temperature for 4.0 hours. Then an equimolar amount of $trenD_2^{2+}$ (β,β',β''-triaminotriethylamine) was added to destroy $[Zn(H_{-1}ADA)_2^{4-}]$ according to eqn. 4. An infrared spectrum of the resultant solution showed two carbonyl absorptions

$$trenH_2^{2+} + Zn(H_{-1}ADA)_2^{4-} \rightleftharpoons Zn(tren)^{2+} + 2ADA^{2-} \qquad (4)$$

at 1580 cm^{-1} and 1630 cm^{-1} corresponding to ionized carboxylate groups and the amide group in ADA^{2-}.

In order to understand the single broad peak in spectrum 5, one must account for the greater chemical shift of the amide methylene resonance compared to that of the carboxylate, the loss of relative acidity of both types of methylene resonances, and the broad nature of the peak. The first can be explained by deprotonation of an amide group, the second by a change in stereochemistry (eqn. 5) [12], and the third by an exchange reaction (eqn. 6).



(5)

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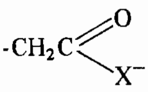
$$[Zn(H_{-1}ADA_1)(ADA_2)^{3-}] \rightleftharpoons [Zn(H_{-1}ADA_2)(ADA_1)^{3-}] \qquad (6)$$

The change in stereochemistry frees three of the four carboxylate groups, resulting in less acidic methylene groups since they are no longer chelated to a positively charged Zn. The ionized amide group with a negatively charged $C(O)NH^-$ group adjacent to the methylene carbon will result in a much less acidic hydrogen atom. The single sharp peak in spectrum 6 indicates the formation of $[Zn(H_{-1}ADA)_2^{4-}]$ (III) in which the coordinated deprotonated amide methylene groups absorb at the same frequency as the uncoordinated methylene groups of the carboxylate moieties. The large chemical shift observed in spectrum 4 as compared to 5 is primarily a result of the change in stereochemistry (eqn. 5), while there is little change in chemical shift between spectra 5 and 6 for one type of



$$-CH_2C\begin{matrix}\diagup O \\ \diagdown X^-\end{matrix}$$

group is simply exchanged for another. The sharpness of the peak now indicates that all four carboxylate methylene groups are equivalent as are the two amide methylene groups (III) (no exchange reaction).

Conductometric titrations from a = 2.0 (3.0, NTA) (Fig. 2) of $[Zn(ADA)_2^{2-}]$, $[Zn(IMDA)_2^{2-}]$ [3], and $[Zn(NTA)_2^{4-}]$ [3] are shown in Fig. 2. It is quite obvious that no chemical reaction is occurring above a = 2.0 (a = 3.0) in the latter two cases. However, the curve for $[Zn(ADA)_2^{2-}]$ doesn't approach a straight line until a = 2.80, suggesting that base is reacting with the metal complex. Hydroxo complex formation can be ruled out since the donor groups in $[Zn(IMDA)_2^{2-}]$, $[Zn(NTA)_2^{4-}]$, and $[Zn(ADA)_2^{2-}]$ (I) are probably equivalent. Therefore, the conductance data confirms amide deprotonation reactions as suggested by the NMR data.

The fact that $[Zn(ADA)_2^{2-}]$ undergoes amide deprotonation more easily than $[Co(ADA)_2^{2-}]$ (Table

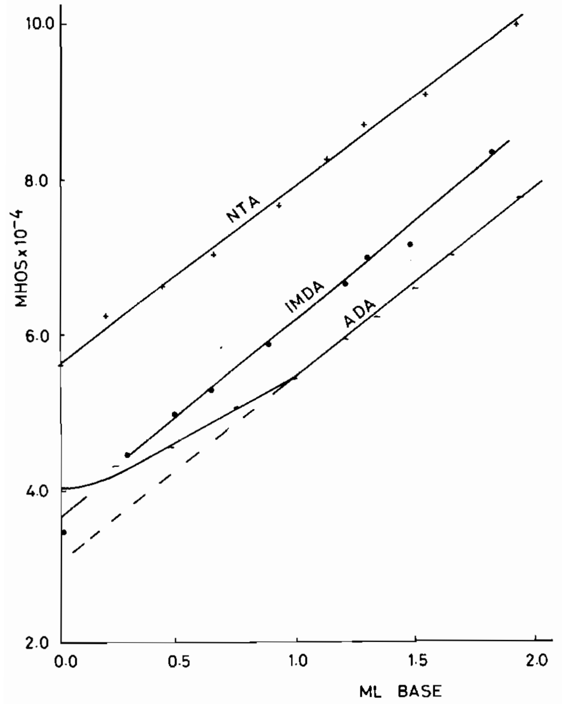


Fig. 2. Conductometric titrations of $[Zn(IMDA)_2^{2-}]$ and $[Zn(ADA)_2^{2-}]$ from a = 2.0 to a = 3.5 and that of $[Zn(NTA)_2^{4-}]$ from a = 3.0 to a = 4.5. Dashed lines are an extrapolation of the linear portion of the ADA curve.

I) is probably due to the Zn(II) stereochemical change (eqn. 5). The coordination sites of tetrahedral Zn(II) should be more acidic than those of an octahedral geometry [12]. Since Martin [7] has shown that deprotonated bis(dipeptide)cobalt(II) chelates are octahedral with a high spin-low spin equilibrium mixture, the paring energy and the increase in ligand field stabilization energy in these metal chelates are roughly equivalent. Apparently, the increased acidity of Zn(II) (Oh → Td) upon amide ionization more than compensates for the partial energy gain by the Co(II) system upon going from high to low spin.

The discovery of Zn(II) induced amide deprotonations is important in that previous arguments that amide and presumably other deprotonation reactions primarily are a result of large increases in ligand field stabilization energies due to increased σ-donor strengths of the ionized groups are apparently spurious; for Zn(II), a d^{10} system, can not possibly attain increased ligand field stabilization. This is not to say, however, that such stabilization does not, however, provide an 'extra' driving force which allows Cu(II) and Ni(II) to deprotonate amide groups below pH 7 [8, 9], in contrast to Co(II) and Zn(II) which promote the same reactions only at high pH. Apparently only a positively charged center is needed to induce amide deprotonations. Furthermore, previous studies *e.g.*, that of Zn(II) complexes of gluthathione by Rabenstein [14], and of glycylhistidine by Martin [15], should be more convincing now that Zn(II) has been shown to induce such deprotonation reactions at pH 10 in a more simple system.

Acknowledgements

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6 Visible spectra at various a values were obtained on a Cary 14 R spectrophotometer using a set of matched quartz 1 cm cells. The λ_{max} and ϵ_{max} values for $Co(H_{-1}ADA)_2^{4-}$ are 625(96), 600 sh (88) and 505(68), 480 sh (62) where λ_{max} is in nm, ϵ_{max} in M^{-1} cm^{-1}, and sh is shoulder. The spectrum is very similar to that of deprotonated bis(glycylglycinato)cobalt(II) [7]. The λ_{max} are 610(23), 590 sh (18), 480(12) and 470 sh (11). There is no peak in the 4000–8000 cm region, indicating that $Co(H_{-1}ADA)_2^{4-}$ is not tetrahedral.
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12 As stated earlier, deprotonated amide groups are powerful σ-donors and induce stereochemical changes in Ni(II) chelates (Oh to square planar) and spin change in Co(II) (high to low). Weak σ-donors yield Oh Zn(II) complexes in aqueous solution (H_2O, Cl^-) while strong σ-donors often yield tetrahedral Zn(II) complexes (CN^-). F. A. Cotton and G. Wilkinson, 'Advanced Inorganic Chemistry', 2nd Ed., Wiley, New York, p. 607 (1966).
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