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Stability of Bivalent Metal Complex With L-Hydroxyproline

Inamul-Haq

School of Environmental Sciences, Jawaharlal Nehru University,
New Delhi 110067, India

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Potentiometric titration of L-hydroxyproline with NaOH solution at 30° ± 0.1 °C in a medium of constant ionic strength, $\mu = 0.1M$ (KNO_3) gave the stepwise formation constants of the complexes formed between Mn(II), Co(II) and Zn(II) ions. The values were $\log K_1 = 3.45$; $\log K_1 = 4.58$, $\log K_2 = 4.03$; $\log K_1 = 5.08$, $\log K_2 = 4.66$; for Mn(II), Co(II) and Zn(II) complexes. The order of stability constant is in accordance with the *Irving-Williams* series.

[Keywords: Hydroxyproline, Co(II), Mn(II), Zn(II) *complexes; Transition metal complexes of hydroxyproline]*

Die Stabilität bivalenter Metall-Komplexe mit L-Hydroxyprolin

Potentiometrische Titration von L-Hydroxyprolin in NaOH-Lösung bei $30 \pm 0{,}1$ °C und einer konstanten Ionenstärke von $\mu = 0{,}1M$-KNO_3 ergab stufenweise die Bildungskonstanten der entsprechenden Komplexe mit Mn(II), Co(II) und Zn(II). Die Werte sind $\log K_1 = 3{,}45$; $\log K_1 = 4{,}58$, $\log K_2 = 4{,}03$; $\log K_1 = 5{,}08$, $\log K_2 = 4{,}66$; für Mn(II); Co(II); Zn(II). Die Reihenfolge der Stabilität der Komplexe ist im Einklang mit der *Irving-Williams*-Reihung.

Introduction

Recent studies[1,2] have shown that organic compounds containing coordinating groups such as $—NH_2$, —SH, —COOH and =N. reduce radiation damage in an exposed organism. They probably reduce radiation damage in the system by forming stable chelates with metal ion responsible for damage. It has also been pointed out that metal complexes which can bypass normal proteins and are able to release metal ions preferentially complexing with sites on virus proteins, may prove effective in deactivating the offending protein molecule[3]. The precise role of metal ions in such complicated system has not been well understood. However, an insight into this can be obtained by studying

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the equilibria involved between metal ions and amino acids. Therefore L-hydroxyproline and three biologically important metal ions have been taken up to study the physical properties of these transition metal ions with respect to the chosen ligand. From a literature survey it was found that metal complexes of L-hydroxyproline have been very little investigated[4–6]. *Perrin*[7] determined the composition and stability constants of 1:1 ferrous-hydroxyproline complexes by the *Bjerrum* method as developed by *Calvin* and *Wilson*, using least square treatment. Molar electrode potentials for the $Fe^{+3}:Fe^{+2}$ hydroxyproline complexes were calculated. A study on metal complexes of hydroxyproline with manganese, cobalt and zinc has not yet been reported. In continuation of the previous work[8–11], the present paper deals with the determination of the stepwise stability constants of these complexes (potentiometrically, using least square treatment).

Experimental

Reagents

Analytical reagent grade chemicals were used if available. $0.01M$-zinc nitrate, manganese nitrate and cobaltous chloride were prepared and standardised by sodium salt of EDTA (BDH, AnalaR).

pH-*Measurement*

pH measurements were made with a ELICO pH-meter model L1-12, a glass electrode and a saturated calomel reference electrode at 30 °C in an ionic background of $0.1M$-KNO_3. The initial standardisation of the pH meter was made with a buffer of pH 4.0 and pH 7.0 prepared by dissolving buffer tablet (BDH).

Potentiometric Titration

Potentiometric titrations were carried out in a 100 ml glass vessel. Gentle stirring and exclusion of CO_2 was achieved by bubbling oxygen-free nitrogen through the solution. The mole ratio of metal to ligand was kept 1:5 in order to fulfil the maximum coordination number of the metal. In order to evaluate the protonation constant and metal-ligand stability constants by the *Bjerrum-Calvin*[12,13] method as modified by *Irving* and *Rossotti*[14], the solutions prepared from the following amounts of reagents were titrated with standard carbonate free $0.2M$-KOH: Mixture (a): acid (10 ml of $0.5M$-KNO_3 and 5 ml of $0.01M$-HNO_3), (b): ligand [mixture (a) plus 10 ml of $0.025M$-hydroxyproline], (c): complex [mixture (b) plus 5 ml of $0.01M$-metal solution]. The total volume was made up to 50 ml by double distilled water. Mixture (a), (b) and (c) were separately titrated at least twice to check reproducibility. The ionic strength was maintained at $\mu = 0.1M$ by KNO_3 solution. The initial concentrations of KNO_3, HNO_3, ligand and metal were $0.1M$, $0.001M$, $0.005M$ and $0.001M$ respectively. The plots of pH versus the volume of the alkali required to obtain the corresponding pH change were plotted. The shapes of the titration curves were as usual.

Results and Discussion

At low pH the nitrogen atom of the ligands protonates; as the pH rises, this proton is lost, and it is followed by those from the hydroxyl group, leaving a negative centre for complex formation.

For the formation of the protonated and complex species HL, H_2L, ML and ML_2. The following equations apply:

(A) The degree of formation of ligand proton species

$$n_{\bar{A}} = 1 - \frac{(V_2 - V_1)\,(N + E_0)}{(V_0 + V_1)\,T_L^{\circ}}$$

where N = molarity of the standard KOH, E_0 = initial molarity of HNO_3, T_L° = initial total concentration of ligand and V_0 = initial total volume. V_1, V_2 and V_3 are the volumes of NaOH solution giving the same pH meter reading in the three titrations above.

(B) Likewise, the values of n^-, the average number of ligands attached per metal ion and the free ligand exponent (pL) are calculated from the following equations:

$$n^- = \frac{(V_3 - V_2)\,[N + E_0 + T_L^{\circ}\,(1 - n_{\bar{A}})]}{(V_0 + V_2)\,n_{\bar{A}}\,T_M^{\circ}},$$

where T_M° = initial total concentration of metal, and

$$pL = -\log[L] = \log\left(\frac{1 + [H^+]KH_1 + [H^+]^2 KH_1\,KH_2}{T_L^{\circ} - n^-\,T_M^{\circ}}\right)$$

where KH_1 and KH_2 are the proton ligand stability constants. The second term in this equation was neglected ($\log KH_2$ very small[15] and calculating pL under this condition). The value of $n_{\bar{A}}$ lies between 0 and 1, showing that only L and HL are present in solution.

Thus, when $0 < n_{\bar{A}} < 1$, then equilibrium

$$L^- + H^+ \overset{KH_1}{\rightleftharpoons} LH$$

is involved, and

$$n_{\bar{A}} = \frac{C_H - [H^+]}{C_L} = \frac{[HL]}{[L] + [HL]} = \frac{KH_1\,[H^+]}{1 + KH_1\,[H^+]}$$

Therefore, $\log KH_1 = \mathrm{pH} + \log \dfrac{n_{\bar{A}}}{(1 - n_{\bar{A}})}$.

The value of $\log KH_1$ was obtained from the plot of pH vs. log $n_{\bar{A}}/(1 - n_{\bar{A}})$ which was a straight line having a slope equal to unity, indicating that one proton was dissociated. The intercept of this plot gave the value of $\log KH_1$ as 9.34. The same value has been obtained, by the *Bjerrum* half integral method, from the plot of $n_{\bar{A}}$ versus pH. This value was found in good agreement with the value reported in the literature[15, 16]. From the titration curves using solution (a), (b) and (c), n^- values of the metal complexes were determined at various pH values. From these data, the corresponding pL values were calculated.

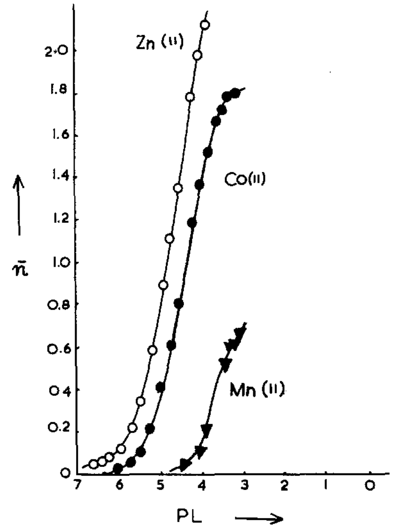

Fig. 1. Formation curves for metal complexes of L-hydroxyproline

The formation curves (Fig. 1) of the metal-ligand system shows that the value of n^- exceeds 1.5 for Co(II) and Zn(II) complexes indicating thereby the formation of 1:1 and 1:2 complexes. For Mn(II) complexes the study reveals the formation of 1:1 complexes which might be due to hydrolysis. The best values of stepwise stability constants of these complexes were computed by a least square method using the equation given by *Irving* and *Rossotti*[14].

The plot of $\frac{n^-}{(1-n^-)[L]}$ vs. $\frac{(2-n^-)[L]}{(1-n^-)}$ was a straight line (Fig. 2*a*). The slope and intercept of these plots gave the values of K_1K_2 and K_1, respectively, which were used for calculating the values for $\log K_1$ and $\log K_2$. In the case of manganese complexes this plot was found to be a straight line parallel to the x-axis the intercept providing the value for

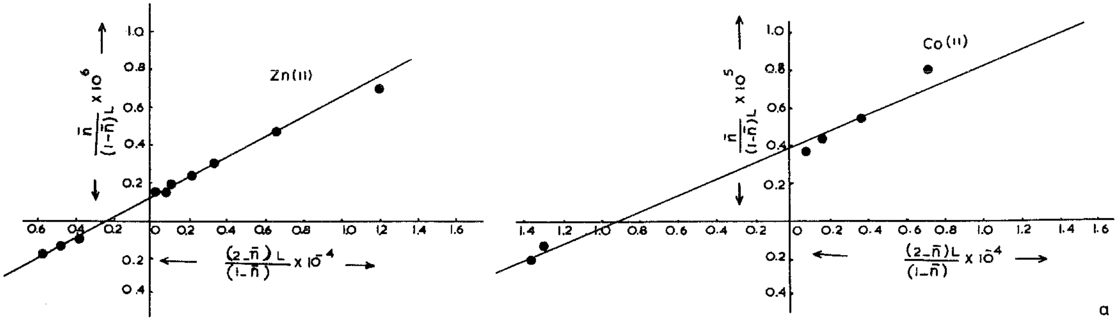

Fig. 2*a*. Computation of $\log K_1$ and $\log K_2$ for Zn(II) and Co(II) complexes of L-hydroxyproline by least square treatment

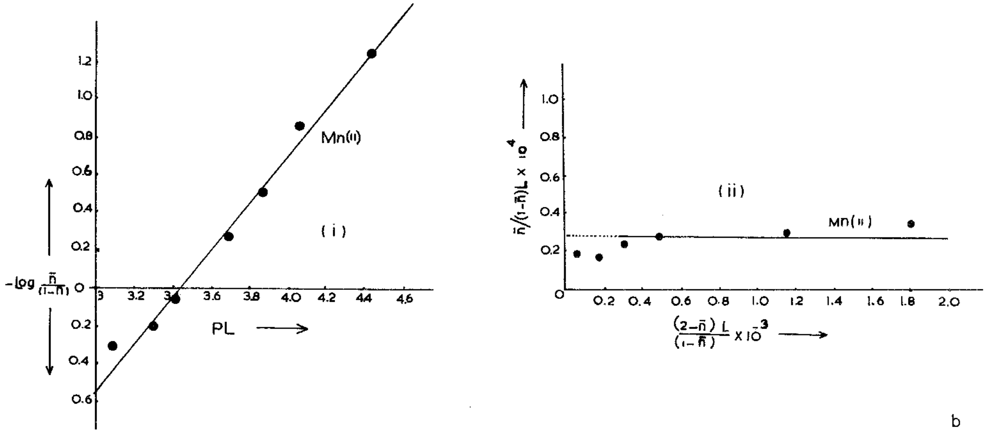

Fig. 2*b*. Computation of $\log K_1$ of Mn(II)—L-hydroxyproline complex: (i) plot of $\log \bar{n}/(1-\bar{n})$ vs. pL, (ii) plot of $(2-\bar{n})L/(1-\bar{n})$ vs. $\bar{n}/(1-\bar{n})L$

Table 1. *Values of the proton-ligand and metal-ligand stability constants of L-hydroxyproline*
[solvent: water; $\mu = 0.1M$-KNO_3, $t = 30 \pm 0.1\,°C$]

Metal	$\log K_1$	$\log K_2$	$\log B_2$
H^+	9.34[a]	—	—
Mn^{+2}	3.45	—	—
Co^{+2}	4.58	4.03	8.61 (8.60)[b]
Zn^{+2}	5.08	4.66	9.74 (9.70)[b]

[a] Amino functional group.
[b] This value was obtained from the expression $\log \beta_2 = 2pL$ (at $n^- = 1$).

Table 2. *Values of* pL, n^- exp., n^- calc. *and standard deviations for Zinc(II), Cobalt(II) and manganese(II) complexes of L-hydroxyproline*

Zn(II):									
pL	5.660	5.472	5.400	5.196	5.100	4.650	4.581	4.500	4.400
n^- exp.	0.2270	0.3490	0.4100	0.5970	0.700	1.2800	1.3600	1.4600	1.5800
n^- calc.	0.2448	0.3614	0.4170	0.6096	0.7168	1.2738	1.3524	1.4380	1.5450
				$\sigma = \pm 0.0163$					
Co(II):									
pL	5.646	5.451	5.161	4.880	4.699	3.992	3.818	3.556	
n^- exp.	0.0660	0.1160	0.2330	0.4250	0.6180	1.3730	1.5250	1.6780	
n^- calc.	0.0828	0.1271	0.2351	0.4093	0.5656	1.3569	1.5218	1.7097	
				$\sigma = \pm 0.0240$					
Mn(II):									
pL	3.874	3.700	3.426	3.300	3.092				
n^- exp.	0.2360	0.3400	0.5230	0.6140	0.6680				
n^- calc.	0.2718	0.3580	0.5122	0.5839	0.6938				
				$\sigma = \pm 0.0256$					

K_1. The same value of stability constant of Mn(II) complexes was obtained from the plot of

$$\log \frac{n^-}{(1-n^-)} \text{ vs. } pL$$

(Fig. 2*b*) using the following equation:

$$\log K_1 - \log \frac{n^-}{(1-n^-)} = pL$$

The value of the stability constants are reported in Table 1. A satisfactory agreement between n^{-} exp. and n^{-} calc. values was obtained with standard deviations of 0.025,0.242 and 0.0163, respectively, for Mn(II), Co(II) and Zn(II) complexes indicating high precision of the data (Table 2).

The stabilities of the chelates (taking log K_1 into consideration) were in the order Mn < Co < Zn which is in accordance with the *Irving-Williams* order[17] for bivalent metal ion of the 3d-series. Mn(II) has a greater ionic radius than Co(II) and Zn(II) and hence it formes less stable complexes. The values of the stability constants of metal complexes of proline[18] and values reported in this paper for these metal complexes of hydroxyproline were also compared. It was found that proline complexes are more stable than hydroxyproline complexes because the pK_a of proline is greater than the one of hydroxyproline and therefore the metal ions are bound more firmly by the more basic α-amino group. The preliminary titration studies indicated that the predominating complex of Mn(II) with hydroxyproline was 1:1 which is in agreement with a similar conclusion by *Monk*[19] and *Kroll*[20] in the case of the maganese—proline system. This shows that the mode of binding of hydroxyproline and L-proline[21] with metal ions might be the same.

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