

Metal Complexes of Some Model Peptide Ligands: Part II—Complex Formation of Copper(II) with N-Salicyloyl-*dl*- α -alanine†

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The pH-metric study of the interaction of Cu(II) with N-salicyloyl-*dl*- α -alanine, *o*-(OH)C₆H₄CONHCH(CH₃)COOH, (NSA or LH₂) in 50% (v/v) aq. ethanol at $\mu = 0.2M$ (NaClO₄) and 30 $\pm$ 0.5°C, indicates the formation of [Cu(H₋₁LH)⁰] and [Cu(H₋₁LH)₂⁻] complexes in the pH range 2.5-6.0 followed by deprotonation of [Cu(H₋₁LH)₂⁻] to [Cu(H₋₁L)(H₋₁LH)³⁻] and finally to [Cu(H₋₁L)₂²⁻] in the pH range 8.25 to 11.75. The successive acid dissociation constants (k_{COOH} , k_{OH}) of the ligand LH₂, stepwise equilibrium constants (K'_1 and K'_2) of the complex formation reactions and stepwise deprotonation constants (k_{2OH} , k_{1OH}) of the 1:2 complex ion Cu(H₋₁LH)₂⁻ have been determined. Evidence for bidentate (O, N) chelation of the ligand through its carboxylate O-atom and deprotonated N-peptide atom is provided.

The interactions between metal ions and amino acids and peptides represent as models for metal-protein interactions, specially in biological systems. The present note describes the results of pH-metric study of the reaction of Cu(II) ion with the model peptide ligand N-salicyloyl-*dl*- α -alanine *o*-(HO)C₆H₄CONHCH(CH₃)COOH, (hereinafter NSA or LH₂).

The ligand NSA was obtained by the acid hydrolysis of N-(acetylsalicyloyl)-*dl*- α -alanine¹, purified by recrystallisation from hot water and dried *in vacuo*, m.p. 155° [Found: C, 57.9; H, 5.4; N, 6.6; Eq. wt, 210. *o*-(HO)-C₆H₄CONHCH(CH₃)COOH requires C, 57.4; H, 5.3; N, 6.7%; Eq. wt, 209]. All other reagents were either of AR quality or properly purified and their solutions were made in doubly distilled CO₂-free water and purified dry ethanol². pH measurements were made with a portable Cambridge pH-meter having a glass electrode of 1-13 pH range and a saturated calomel electrode connected to the cell by means of an agar-2(*M*) NaNO₃ bridge. The meter was calibrated using standard 0.01 *M* HClO₄, phthalate buffer and borate buffer solutions with appropriate temperature corrections. pH-metric titrations of the following solutions (total vol. 50 ml): (a) = 0.01 *M* HClO₄; (b) = (a) + 0.01 *M* LH₂; (c) = (b) + 0.001 *M*

Cu(ClO₄)₂; (d) = (b) + 0.002 *M* Cu(ClO₄)₂; and (e) = (a) + 0.01 *M* benzoylglycine were performed in 50% (v/v) water-ethanol medium against a standard NaOH solution in the same solvent medium, maintaining a constant ionic strength, $\mu = 0.2M$, by adding requisite amounts of NaClO₄ at 30 $\pm$ 0.5°C (thermostated). The values of $\bar{n}_H$, $\bar{n}$, $\bar{n}_{OH}$, pLH_2 etc. required for the evaluation of the equilibrium constants were calculated following the procedure of Irving and Rossotti³.

The pH titration curve of solution (b) showed two well-separated buffer regions, in the pH ranges $\approx$ 2.5 to 6.0 and 8.0 to 12.0, corresponding to the deprotonation of the —COOH and the phenolic —OH groups respectively (Fig. 1, curve 3). One equivalent of base per mol of the ligand was consumed in each of the two buffer regions. That the second proton was lost from the phenolic hydroxyl group and not from the peptide bond, was concluded from the pH titration curve of solution (e), wherein benzoylglycine acted as a monobasic acid in the whole pH range studied (Fig. 1, curve 2). The pK_{COOH} and pK_{OH} values have been evaluated and refined by the usual procedure⁴ and are stated in Table 1.

pH titration curves of the solutions (c) and (d) showed two buffer regions, one in the pH range 2.5 to 6.0 in which four mol of base per mol of the metal ion

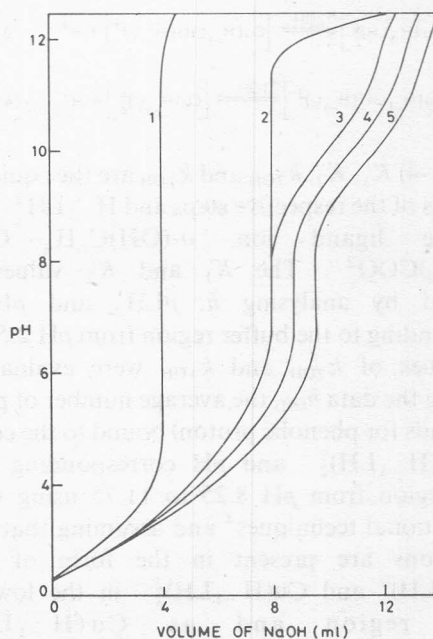


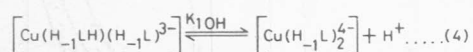
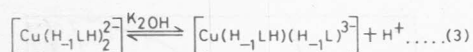
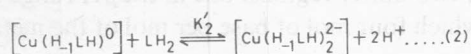
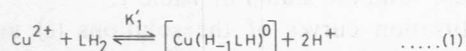
Fig. 1—pH-titration curves of solutions (a), (b), (c), (d) and (e) (see text) at $\mu = 0.2M$ (NaClO₄) and 30 $\pm$ 0.5° against 0.125 *M* NaOH in 50% (v/v) aq. ethanol.

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Table 1—Proton Ligand Constants and Formation Constants of Cu(II) Complexes of N-salicyloyl-dl- α -alaninate[Solvent medium: 50% (v/v) aq. ethanol; $\mu = 0.2M$ (NaClO₄); temp. = $30 \pm 0.5^\circ C$]

Proton-ligand constants		Copper(II)-Ligand constants					
pK_{COOH}	pK_{OH}	pK'_1	pK'_2	pK_{2OH}	pK_{1OH}	$\log \beta_{111}$	$\log \beta_{122}$
4.52	9.76	6.57	6.78	8.87	10.88	7.71	15.21
± 0.04	± 0.04	± 0.04	± 0.04	± 0.04	± 0.04	± 0.04	± 0.04

were consumed and the other in the pH range 8.25 to 11.75 where two mol of base per mol of the metal ion were consumed (Fig. 1, curves 4 and 5). The higher pH buffer region was practically the same as in the case of titration of the ligand NSA in the absence of Cu(II). The liberation of four mol of acid at the early stages of the reaction at pH < 6.0 may be attributed to the coordination of deprotonated —COOH group and N-(peptide) atom of the ligand, ultimately leading to the formation of a 1:2 complex, $Cu(H_{-1}LH)_2^{2-}$ ion, according to the reactions (1) and (2). This was followed by successive deprotonation of the phenolic —OH groups of the two coordinated ligands in the pH range 8.25 to 11.75 according to the reactions (3) and (4):



In Eqs (1-4) K'_1 , K'_2 , k_{2OH} and k_{1OH} are the equilibrium constants of the respective steps and $H_{-1}LH^{2-}$ stands for the ligand ion $o-(OH)C_6H_4-CON-CH(CH_3)COO^{2-}$. The K'_1 and K'_2 values were evaluated by analysing $\bar{n}$, pLH_2 and pH data corresponding to the buffer region from pH 2.5 to 6.0. The values of k_{2OH} and k_{1OH} were evaluated by analysing the data $\bar{n}_{OH}$, the average number of protons (OH stands for phenolic proton) bound to the complex ion, $Cu(H_{-1}LH)_2^{2-}$ and pH corresponding to the buffer region from pH 8.25 to 11.75 using various computational techniques⁴ and assuming that all the Cu(II) ions are present in the form of Cu^{2+} , $Cu(H_{-1}LH)^0$ and $Cu(H_{-1}LH)_2^{2-}$ in the lower pH buffer region and as $Cu(H_{-1}LH)_2^{2-}$, $Cu(H_{-1}LH)(H_{-1}L)^3-$ and $Cu(H_{-1}L)_2^{4-}$ in the higher pH buffer region. Refined pK'_1 , pK'_2 , pK_{2OH} and pK_{1OH} values are listed in Table 1.

Concentrations of the various species involved in the complexation equilibria at different pH values were calculated and the space distribution curves were constructed. At pH around 6.0 practically all the Cu(II) ions were found to exist in the form of the 1:2 complex species $Cu(H_{-1}LH)_2^{2-}$ and the complexes $Cu(H_{-1}LH)^0$ and $Cu(H_{-1}L)(H_{-1}LH)^3-$ were present to a negligible extent. Therefore, the assumption that the complexation steps (1) and (2) were not overlapping with the deprotonation steps (3) and (4), made in the evaluation of the constants K'_1 , K'_2 , k_{2OH} and k_{1OH} was justified.

The overall stability constants^{5,6} β_{111} and β_{122} were calculated from the relations:

$$\beta_{111} = K'_1 / (k_{COOH} \cdot k_{OH})$$

$$\beta_{122} = K'_1 K'_2 / (k_{COOH} \cdot k_{OH})^2$$

The refined $\log \beta_{111}$ and $\log \beta_{122}$ values are given in Table 1.

Although the possibility of enolization of the peptide bond prior to deprotonation can not be ruled out, metal ions such as Cu(II), Ni(II) and Co(II) would prefer to bind with the N-(peptide) atom rather than the O-(peptide) atom because of comparatively stronger ligand field of an N-(peptide) atom. Coordination of amide deprotonated peptides to Cu(II), Cu(III), Ni(II) has been well established⁷⁻¹².

A 1:1 Cu-ligand complex was isolated as a dihydrate on mixing equimolar (0.01M) solutions of the ligand NSA and CuSO₄ and adjusting the pH to ≈ 4.5 i.e. in the pH region where two mol of the base per mol of Cu(II) were consumed. Thus 1:1 complex gave an intense violet colour with neutral FeCl₃ and a monoacetyl derivative also as a dihydrate. The monoacetyl derivative exhibited in its IR spectrum a broad band (1680-1700 cm⁻¹) characteristic of acetyl group. Therefore the isolated 1:1 Cu-ligand complex was formed through bidentate (O, N) chelation i.e., supporting coordination of COOH and N-(peptide) atom after deprotonation, the phenolic —OH group remaining free. In other words the 1:1 complex was $Cu(H_{-1}LH) \cdot 2H_2O$. This was supported by its analytical data and the loss of water molecules at 105°C [Found: Cu, 20.4; N, 4.5; loss at 105°C, 11.26%. $Cu(H_{-1}LH) \cdot 2H_2O$ requires Cu, 20.7; N, 4.6; H₂O, 11.74%]. The monoacetyl derivative could be formulated as $Cu(H_{-1}LOCCH_3) \cdot 2H_2O$ [Found: Cu, 18.3; N, 3.9. $Cu(H_{-1}LOCCH_3) \cdot 2H_2O$ requires Cu, 18.2; N, 4.0%]. The symbol H_{-1} in the above formulae stands for the peptide proton, which was titrable only in the presence of metal ions and H stands for the phenolic proton which was titrable even in the absence of metal ions.

Deprotonation of the phenolic hydroxyl groups of the free and the coordinated ligands in the same buffer

region ($\approx pH$ 8 to 12) indicates nonattachment of this group to Cu(II) in the formation of the complex $Cu(H_{-1}LH)_2^{2-}$ ion and also during its deprotonation. Moreover, due to the inherent preference of $Cu^{2+}(d^9)$ for a square planar structure over an octahedral one, the formation of the complex $Cu(H_{-1}LH)_2^{2-}$ ion will be more favourable through bidentate (O, N) chelation rather than a tridentate (O, N, O) chelation inspite of more favourable entropy effect in the latter case. Further, the π -electron delocalization in the peptide bond demands coplanarity of the sequence $O \cdots C \cdots N \cdots Cu \cdots N \cdots C \cdots O$. Such an effect keeps the phenyl rings coplanar with the peptide bonds, thereby rendering the phenolic $-OH$ group inaccessible to Cu(II). Slightly higher value of pK_{1OH} of the complex $Cu(H_{-1}L)(H_{-1}LH)^{3-}$ as compared to the pK_{OH} of the ligand ion (LH^-) indicates extra stabilization of the phenolic proton in this complex. The extra stabilisation may be provided by the accumulation of negative charge on the metal due to O -(carboxylate) $\rightarrow$ metal and N -(peptide) $\rightarrow$ metal sigma bondings for which pK_{2OH} of $Cu(H_{-1}LH)_2^{2-}$ is slightly lower than pK_{OH} of LH^- , coupled with some back donation through the delocalized π -system of the peptide bond. Hydrogen bonding of the phenolic proton with the carbonyl oxygen of the peptide bond may also contribute to the extra stabilization.

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