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Potentiometric Studies of Mixed Copper(II) Complexes with δ-Aminolevulinic Acid and Some Selected Peptides

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Ternary complex formation in the system Cu(II)-ALA-L (ALA = δ-aminolevulinic acid drug; L = peptides) has been studied by pH-potentiometrically at 25 ± 0.1 ºC with I = 0.10 mol L^{-1} NaCl. The stability constants of binary and ternary complexes were calculated and interpretated. The concentration distribution of the complexes in solution was graphically presented using the HySS program. In addition, the values of Δlog K, log X and relative stabilization percentage (% R.S.) for the mixed-ligand complexes studied have been evaluated and discussed.

Keywords: Solution equilibrium, Potentiometric methods Distribution diagrams Copper(II), Peptides.

INTRODUCTION

δ-Aminolevulinic acid (ALA) is an important compound in the biosynthesis of porphyrins [1,2]. It is employed in neuro-surgical procedures to visualize tumorous tissues [3,4]. Studies from 2006 onwards have determined that the use of this guiding technique intraoperatively might decrease the residual volume of tumor [5,6]. Moreover, rat hair growth was induced as a result of formation of complex Fe^{3+} ion and ALA. Therefore, Fe^{3+}-ALA complex may potentially become a new beneficial remedy for alopecia [7]. Also Cu(II)-tripeptide complex work as a growth factor for hair. One of the tripeptide-copper complexes, which is Cu^{2+}-L-alanyl-L-histidyl-L-lysine encourages human hair follicles growth that is caused as a result of stimulating the preclusion [8]. Most physiological activities concerning the interactions of nucleic acid are promoted by metal ions by ternary (mixed-ligand) complexes formation [9,10]. When a metal ion is found in a solution that contains two or more different ligands, it is possible for ternary (mixed-ligand) complexes as well as several simple ones to be formed, which is determined by pH of the system. The actual formation of the complex is dependent on the relative concentration and the cognation of M^{+} ions across the different ligands present. Amongst other transition metals, Cu(II) delivers vigorous center in various enzymes. In the present investigation, an interaction between δ-aminolevulinic acid drug (ALA) and peptides (L) with Cu(II) was studied at 25 ± 0.1 ºC with ionic strength I = 0.10 mol L^{-1} NaCl using the potentiometric technique. The values of log X, Δlog K and relative stabilization percentages (% R.S.) for ternary complexes examined have been estimated. Ultimately, the distribution of species in solution was also evaluated.

EXPERIMENTAL

All the chemicals and ligands used in this present work were analytical grade purity of Sigma-Aldrich products. Stock solutions of $CuCl_2 \cdot 2H_2O$, HCl and NaCl were prepared in de-ionized water. A solution of δ-aminolevulinic acid (ALA) was prepared with two equivalents of HCl. The ionic strength of each solution was adjusted to 0.1 mol L^{-1} by addition of NaCl as supporting electrolyte. The sodium hydroxide (titrant) solution (CO_2 free) was standardized potentiometrically with potassium hydrogen phthalate (Merck Chem. Co.).

Equilibrium measurements: Using the digital pH meter Griffin p-J-300-010 G at 25 ± 0.1 ºC, pH titrations were performed in a double-walled glass container. Furthermore, ternary system titrations were conducted on aliquots (50 mL) of a low concentration solution (0.005 mol L^{-1}) corresponding Cu(II), δ-aminolevulinic acid drug (ALA) and peptide ligands (L) in 1:1:1 ratio with a known value for standard NaOH volume.

Quantitatively, stability constants (β_{malh}), of ternary complexes equilibrium can be given as:

$$mM + aALA + lL + h\,H \rightleftharpoons M_m\,ALA_a\,L_l\,H_h \quad (1)$$

$$\beta_{malh} = \frac{[M_mALA_aL_lH_h]}{[M]^m[ALA]^a[L]^l[H^h]} \quad (2)$$

(charges omitted for simplicity)

where [M], [ALA], [L] and [H] are the concentrations of Cu(II) ion, δ-aminolevulinic acid, peptides and proton, respectively and the stoichiometric constants are m, a, l and h.

Calculations within the pH range 2.5-12.0 (105-110 data; volume of base/pH), which are included in research concerned with potentiometric were measured using HYPERQUAD computer program [11] and the results are given in Table-1. The HYSS program [12] enabled the calculation of concentration of speciation formed in solution over the pH. Therefore, a visual record can be obtained to detect the main complex species found at a particular pH, principally in the physiological range of pH.

TABLE-1
PROTONATION CONSTANTS OF THE LIGANDS IN AQUEOUS MEDIA AT 25.0 °C AND I = 0.10 mol L^{-1} (NaCl)

Ligands	pK's of ligands	
	pK_{a1}	pK_{a2}
Aminolevulinic (ALA)	2.81 (0.01)	9.83 (0.02)
Glycyl-glycine (Gly-Gly)	3.21 (0.05)	8.04 (0.03)
Glycyl-L-alanine (Gly-Ala)	2.70 (0.006)	7.56 (0.01)
Glycyl-L-valine (Gly-Val)	3.27 (0.03)	7.96 (0.02)
Glycyl-L-leucine (Gly-Leu)	4.32 (0.02)	9.13 (0.002)
Glutamine (Glu)	2.17 (0.02)	9.13 (0.01)
Glycinamide (Gly)	1.78 (0.01)	8.06 (0.01)

Standard deviations are given in parentheses.

RESULTS AND DISCUSSION

Ionization constants (pK_a) of ALA: δ-Aminolevulinic acid (ALA) exists in solution as zwitterion forming compound [13]. By using a HYPERQUAD program to analyze the potentiometric data, two deprotonation can be determined for ALA in the range of pH titratable degree at the protonated imino group and carboxylic proton with pK_a values of 9.83 and 2.81, respectively (Table-1). The ionization of ALA in solution take place according to the following equilibrium:

$$H_2ALA^+ \rightleftharpoons HALA + H^+ \quad (3)$$

The ALA ligand species distribution mark H_2ALA^+ as the protonated form and HALA as the zwitterionic form of ALA as shown in Fig.1.

Stability constant of binary copper(II) complex: The mechanism used to lower the values of pH in all potentiometric binary system curves was shifted the buffer region of ligand *via* the use of free ligand solutions. Therefore, releasing of protons from ligands, such as peptides and ALA assists complexation reactions to proceed. More than species of Cu(II)-ALA (1100), Cu(II)-$(ALA)_2$ (1200), Cu(II)-$(ALA)_2$ H (1201), Cu(II)-$(ALA)_2$ H_2 (1202),Cu(II)-ALA (OH) 110-1, Cu(II)-L (1010), Cu(II)-$(L)_2$ (1020), Cu(II)-(L) H_{-2} (101-1) and Cu(II)-$(ALA)_2$ H_{-1} (102-1) complexes were found on the selected form with the best suited statistical. At the amino end of molecule and

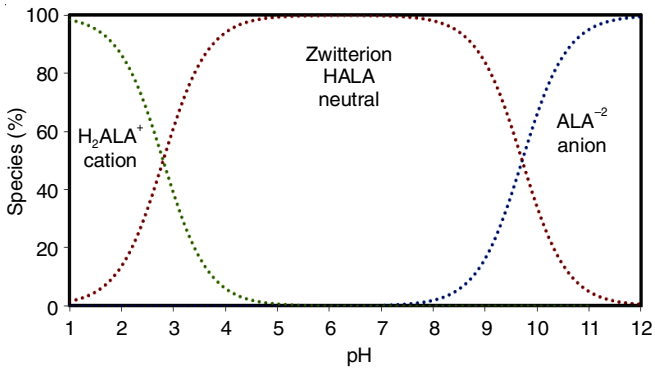

Fig. 1. Distribution curves as a function of pH calculated for ALA system at 25.0 °C and I = 0.10 mol L^{-1} NaCl

with the aid of carbonyl oxygen, chelation in the complexation between Cu(II) and L commences. Finally, chelation continues with the sequential coordination and deprotonation of amide group [14,15]. Fig. 2 depicts the species distribution diagram of Cu(II)-ALA complex and the stability constant values of the species are shown in Table-2.

TABLE-2
STABILITY CONSTANTS OF THE BINARY SPECIES IN THE Cu(II)-ALA OR PEPTIDES SYSTEMS AT 25 °C AND 0.1 mol/L IONIC STRENGTH

Systems	m	a	l	h^n	log β^b
Cu(II)-ALA	1	1	0	0	8.67 (0.003)
	1	2	0	0	15.78 (0.01)
	1	2	0	1	22.90 (0.01)
	1	2	0	2	28.01 (0.02)
	1	1	0	-1	1.56 (0.01)
Cu(II)-Gly-Gly	1	0	1	0	7.60 (0.02)
	1	0	2	0	13.04 (0.01)
	1	0	1	-1	1.34 (0.01)
	1	0	1	-2	-7.76 (0.01)
	1	0	2	-1	4.41 (0.03)
Cu(II)-Gly-Ala	1	0	1	0	8.21 (0.01)
	1	0	2	0	15.15 (0.02)
	1	0	1	-1	1.98 (0.01)
	1	0	1	-2	-6.78 (0.01)
	1	0	2	-1	3.72 (0.01)
Cu(II)-Gly-Val	1	0	1	0	8.38 (0.01)
	1	0	2	0	16.05 (0.001)
	1	0	1	-1	0.43 (0.01)
	1	0	1	-2	-6.98 (0.001)
	1	0	2	-1	3.67 (0.01)
Cu(II)- Gly-Leu	1	0	1	0	8.21 (0.01)
	1	0	2	0	16.10 (0.01)
	1	0	1	-1	1.56 (0.002)
	1	0	1	-2	-7.04 (0.001)
	1	0	2	-1	4.55 (0.01)
Cu(II)-Glu	1	0	1	0	7.81 (0.01)
	1	0	2	0	13.63 (0.01)
	1	0	1	-1	1.06 (0.01)
	1	0	2	-1	3.92 (0.01)
Cu(II)-Gly	1	0	1	0	4.27 (0.01)
	1	0	2	0	9.33 (0.01)
	1	0	1	-1	0.48 (0.03)
	1	0	1	-2	-5.61 (0.001)

[n]m, a, l and h represents stoichiometric constants corresponding to Cu(II), ALA, L and H^+, respectively. [b]Standard deviations are given in parentheses and coefficient –1 designate proton loss.

Stability constant of ternary copper(II) complexes: Stability constant values of binary Cu(II) complexes with ALA or peptides (Table-2) are the same order, therefore the ligation

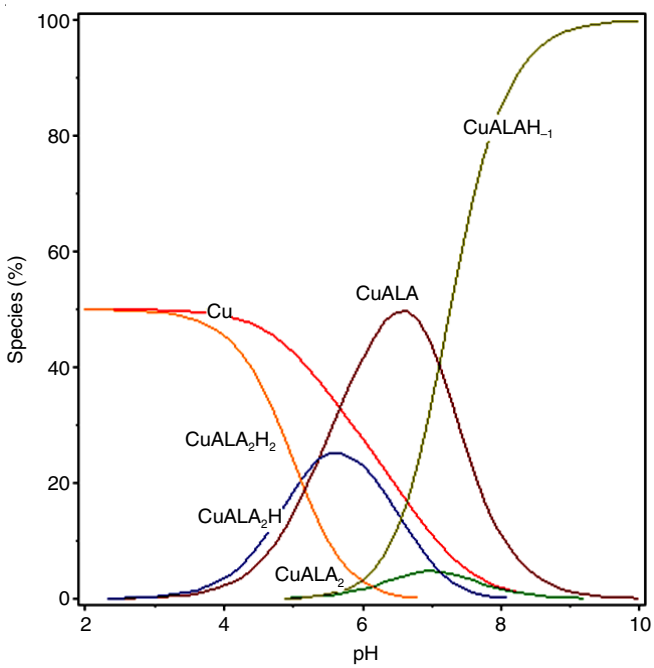

Fig. 2. Distribution curves as a function of pH calculated for Cu(II)-ALA system at 25 °C and 0.10 mol L^{-1} ionic strength

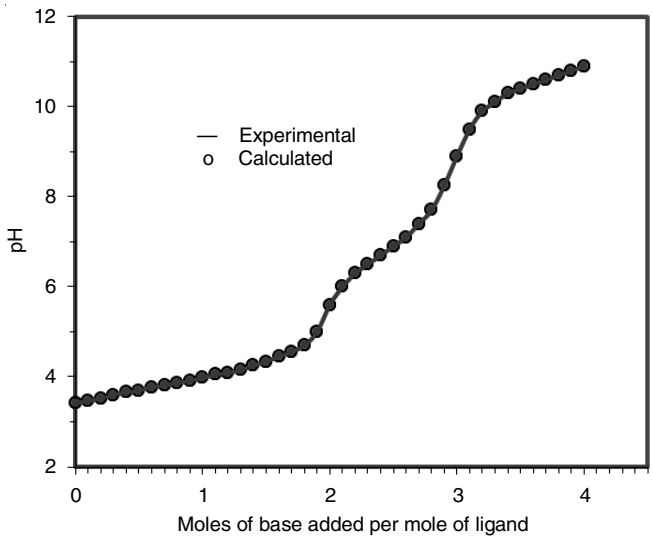

Fig. 3. Potentiometric titration curves for Cu(II)-ALA-Gly-Gly system at 25 °C and 0.10 mol L^{-1} ionic strength

of ALA and peptides with copper(II) will proceed simultaneously. Verified further evidence of mixed ligand complex formation by agreement and *via* a simultaneous mechanism between the calculated and examined data for the glycyl-glycine system (Fig. 3). Two complex species were formed with stoichiometric coefficients 1110 [Cu(ALA)(L)] and 111-1 [Cu(ALA)(LH_{-1})]. In case of Cu(ALA)(L), the peptide was linked through amino and carbonyl oxygen groups. When pH increased, the coordination sites shall move from carbon oxygen to nitrogen amide. [Cu(ALA)LH_{-1}] complexes were formed through amide groups deprotonation. Moreover, determination of the pK_a values was accomplished using the following equation:

$$pK_a = \log\beta_{1110} - \log\beta_{111-1} \quad (3)$$

The pK_a' values of amide groups for Gly-Gly, Gly-Ala, Gly-Val, Gly-Leu, Glu and Gly were 9.18, 9.16, 9.01, 9.59, 10.80 and 5.66, respectively. When transitioning from species 1110 to 111-1, the potential change in structure may be blocked due to the presence of a bulky substituent group on the peptides, except glycinamide, which allows glycinamide complex to maintain a lower pK_a value in comparison to other peptides.

The Δlog K value for deprotonated ternary complexes formed through simultaneous mechanism are given by eqn. 4, whereas those of the induce deprotonated peptide complex can be calculated using eqn. 5:

$$\Delta\log K = \log\beta_{1110} - \log\beta_{1100} - \log\beta_{1010} \quad (4)$$

$$\Delta\log K = \log\beta_{111-1} - \log\beta_{1100} - \log\beta_{101-1} \quad (5)$$

The values of Δlog K for the mixed ligand complexes studied are listed in Table-3. By compared the Δlog K value of the ternary complexes formed in this study with theoretical Δlog K value for a distorted-octahedral Cu(II) complex [16] found that the Δlog K is greater than -0.9, this should be taken as an indication that ternary complex is favoured.

Percentage of relative stability (% R.S.) was used to measure the stability of a ternary complex, which is represented by eqn. 6:

$$\%R.S. = \left(\frac{\log K^{Cu(ALA)}_{Cu(ALA)L} - \log K^{Cu}_{Cu(L)}}{\log K^{Cu}_{Cu(L)}}\right)\times 100 \quad (6)$$

For all systems, the parameter % R.S. was negative (Table-3). This might be considered as an evidence for the occurrence of

TABLE-3
STABILITY CONSTANTS OF THE TERNARY SPECIES IN THE Cu(II)-ALA-PEPTIDES SYSTEMS AT 25 °C AND 0.1 mol/L IONIC STRENGTH

Systems	m	a	l	h[n]	log β[b]	pK_a	Δlog K	$\log K^{Cu(ALA)}_{Cu(ALA)L}$	$\log K^{Cu(L)}_{Cu(ALA)L}$	%R.S.[c]	log X
Cu(II)-ALA-Gly-Gly	1	1	1	0	13.33 (0.01)	9.18	-2.94	4.66	5.73	-38.68	-2.16
	1	1	1	-1	4.15 (0.01)		-5.86				
Cu(II)-ALA-Gly-Ala	1	1	1	0	13.92 (0.01)	9.16	-2.96	5.25	5.71	-36.05	-3.09
	1	1	1	-1	4.76 (0.01)		-5.89				
Cu(II)-ALA-Gly-Val	1	1	1	0	13.01 (0.02)	9.01	-4.04	4.34	4.63	-48.21	-5.81
	1	1	1	-1	4.00 (0.01)		-5.10				
Cu(II)-ALA-Gly-Leu	1	1	1	0	13.34 (0.01)	9.59	-3.54	4.67	5.13	-43.12	-5.20
	1	1	1	-1	3.75 (0.01)		-6.48				
Cu(II)-ALA-Glu	1	1	1	0	13.83 (0.02)	10.80	-2.65	5.16	6.02	-33.93	-1.75
	1	1	1	-1	3.03 (0.01)		-6.65				
Cu(II)-ALA-Gly	1	1	1	0	10.87 (0.01)	5.66	-2.07	2.20	6.60	-48.48	-3.37
	1	1	1	-1	5.21 (0.001)		-3.94				

[a]m, a, l and h are the stoichiometric coefficient corresponding to Cu(II), ALA, peptides and H^+, respectively. [b]Standard deviations are given in parentheses. [c]Percentage of relative stabilization value. The coefficient –1 refers to loss of H^+ from the coordinated amide group of the peptide.

enhanced stabilities. Negative values of % R.S. agreed with the Δlog K values.

The log X (non-proportional dissociation constant) can be used to describe the formation tendency of ternary complexes. It measures the tendency of 1 mol each of the binary complexes $Cu(ALA)_2$ and $Cu(L)_2$ to disproportionate forming 2 mol of Cu(ALA)L. This constant equilibrium expression was calculated by equilibrium eqns. 7 and 8:

$$Cu(ALA)_2 + Cu(L)_2 \rightleftharpoons 2Cu(ALA)(L) \quad (7)$$

$$\log X = 2\log\beta^{Cu}_{CuALAL} - \left(\log\beta^{Cu}_{CuALA_2} + \log\beta^{Cu}_{CuL_2}\right) \quad (8)$$

where $X = \dfrac{[Cu(ALA)(L)]^2}{[Cu(ALA)_2][Cu(L)_2]}$.

The log X values were calculated (Table-3) and the results showed that the values were higher than that expected on a statistical basis (0.6) [17]. This means that the formation of binary complexes was more prevalent than ternary complexes in these systems. A species distribution diagram obtained for Cu(II)-ALA-glycyl-glycine is shown in Fig. 4. The [Cu(ALA)L] (1110) starts to format pH at ~2.8 and with increasing pH, its concentration increases reaching a maximum of 33% at pH = 5. Another increase in pH was accompanied by a decrease in the concentration of 1110 and an increase in the formation of $[Cu(ALA)LH_{-1}]$ (111-1)].

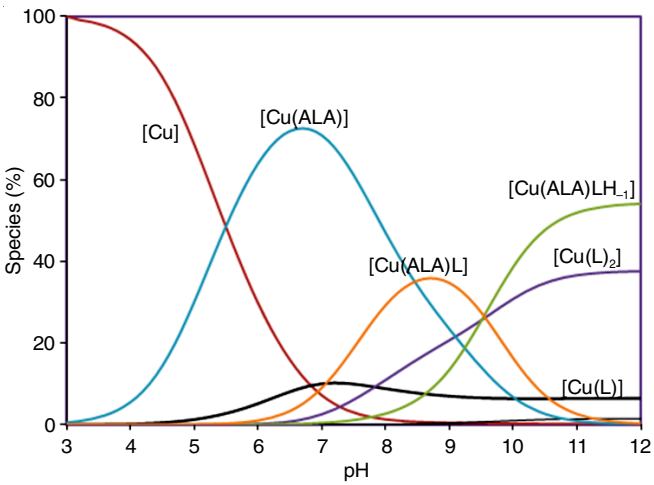

Fig. 4. Distribution curves as a function of pH calculated for Cu(II)–ALA-Gly-Gly system at 25 °C and 0.10 mol L^{-1} ionic strength of NaCl

Conclusion

A potentiometric titration technique has been used to determine the formation equilibria of binary and ternary complexes of Cu(II) with δ-aminolevulinic acid (ALA) as ligand and some selected peptides in aqueous medium at 25 ºC having ionic strength I = 0.10 mol/L NaCl. The order of stability of Cu(II) ion obtained in the mixed ligand complex systems examined in aqueous solution was as follows: Cu(II):ALA:Gly-Val > Cu(II):ALA:Gly-Leu > Cu(II):ALA:Gly-Gly > Cu(II):ALA:Gly-Val. The relative stabilization percentage (% R.S.) values correspond to the Δlog K values were used as a measure of ternary complexes stability.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. L.C. Gardener and T.M. Cox, *J. Biol. Chem.*, **263**, 6676 (1988).
2. D. Von Wettstein, S. Gough and C.G. Kannangara, *Plant Cell.*, **7**, 1039 (1995); https://doi.org/10.1105/tpc.7.7.1039
3. C.G. Hadjipanayis, G. Widhalm and W. Stummer, *Neurosurgery*, **77**, 663 (2015); https://doi.org/10.1227/NEU.0000000000000929
4. I.Y. Eyüpoglu, M. Buchfelder and N.E. Savaskan, *Nat. Rev. Neurol.*, **9**, 141 (2013); https://doi.org/10.1038/nrneurol.2012.279
5. W. Stummer, U. Pichlmeier, T. Meinel, O.D. Wiestler, F. Zanella and H.J. Reulen, *Lancet Oncol.*, **7**, 392 (2006); https://doi.org/10.1016/S1470-2045(06)70665-9
6. I.Y. Eyüpoglu, N. Hore, N.E. Savaskan, P. Grummich, K. Roessler, M. Buchfelder and O. Ganslandt, *PLoS ONE*, **7**, e44885 (2012); https://doi.org/10.1371/journal.pone.0044885
7. Y. Morokuma, M. Yamazaki, T. Maeda, I. Yoshino, M. Ishizuka, T. Tanaka, Y. Ito and R. Tsuboi, *Int. J. Dermatol.*, **47**, 1298 (2008); https://doi.org/10.1111/j.1365-4632.2008.03783.x
8. H.K. Pyo, H.G. Yoo, C.H. Won, S.H. Lee, Y.J. Kang, H.C. Eun, K.H. Cho and K.H. Kim, *Arch. Pharm. Res.*, **30**, 834 (2007); https://doi.org/10.1007/BF02978833
9. R. Singh, S. Tyagi, S. Singh, S.M. Singh and U.P. Singh, *Synth. React. Inorg. Met.-Org. Chem.*, **32**, 853 (2002); https://doi.org/10.1081/SIM-120005607
10. S. Singh and A.K. Ghose, *J. Indian Chem. Soc.*, **73**, 650 (1996).
11. P. Gans, A. Sabatini and A. Vacca, *Talanta*, **43**, 1739 (1996); https://doi.org/10.1016/0039-9140(96)01958-3
12. L. Alderighi, P. Gans, A. Ienco, D. Peters, A. Sabatini and A. Vacca, *Coord. Chem. Rev.*, **184**, 311 (1999); https://doi.org/10.1016/S0010-8545(98)00260-4
13. Y. Ishihama, Y. Oda and N. Asakawa, *J. Pharm. Sci.*, **83**, 1500 (1994); https://doi.org/10.1002/jps.2600831025
14. M.L. Pires dos Santos, A. Faljoni-Alário, A.S. Mangrich and A.M. Costa Ferreira, *J. Inorg. Biochem.*, **71**, 71 (1998); https://doi.org/10.1016/S0162-0134(98)10034-X
15. K. Várnagy, B. Bóka, I. Sóvágó, D. Sanna, P. Marras and G. Micera, *Inorg. Chim. Acta*, **275-276**, 440 (1998); https://doi.org/10.1016/S0020-1693(98)00079-6
16. H. Sigel, *Angew. Chem. Int. Ed. Engl.*, **14**, 394 (1975); https://doi.org/10.1002/anie.197503941
17. R. DeWitt and J.I. Watters, *J. Am. Chem. Soc.*, **76**, 3810 (1954); https://doi.org/10.1021/ja01643a066