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Copper (II) Complexes with Some Essential Amino Acids: Formation Constant Studies In 80% Ethanol-Water Medium

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Abstract

The formation constant of copper (II) ion with some essential amino acids viz. valine, leucine, methionine, phenylalanine and tryptophan as ligand were determined pH metrically at 27°C and an ionic strength of O.1M $NaClO_4$ in 80% (v/v) ethanol-water medium. The calculations of stability constant of have been made using the computerized programmed.

Keywords: Stability constant, logK and pK.

1. Introduction

The metal ions are integral parts of enzymes and play an important role in the biological system, such as to trigger a reaction, control reaction mechanism, stabilize protein structure, maintain structure of cell walls etc. Latest information indicates regulation of metabolism and growth of animal cell is dependent upon the mobilization of divalent and trivalent metal ions. Short resume of relevant biological importance of metal ions is discussed here.

Copper is a transition metal ion and is used by various enzymes in the body in different biochemical reactions. These reactions may be creating energy, decreasing the body's inflammatory blood clotting etc. Copper is absorbed by the body at two main sites such as small intestine and stomach. Copper does not float through the blood stream as copper ion but is carried by proteins. Two main carrier proteins especially for copper are ceruloplasmin and albumin; these can carry many things including copper. Copper is stored in proteins called metallothione. Enzymes are proteins specialized to assist in a chemical function. Copper is needed by enzymes as a helper in a chemical reaction. This function makes copper essential for cytochrome C oxidase, essential for energy and superoxide dismutase essential oxidative tissue damage etc.

In recent years it has been proved that transition metals like copper is essential for normal development and function of human cells. Disruption of copper metabolism causes severe neurodegenerative disease, such as Willson's disease and Menke's disease with symptoms that range from psychiatric abnormalities and motor dysfunction to poor temperature control a liver & kidney abnormalities.

Tryptophan[1] is aromatic essential glycogenic and ketogenic amino acid. In tryptophan metabolism, anthranilic acid finally

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converted to glutaric acid, which gives two molecules of acetate. Tryptophan can form vitamin Niacin. Serotonin is a decarboxylation product of tryptophan, which are a vasoconstrictor, smooth muscle constrictor and cerebral stimulant. Serotonin is formed by intestinal epithelial cells, blood platelets and in the brain. 'Argentaffinomas' is a tumor of intestine produces large amounts of serotonin. Its metabolite product 5-Hydroxyindole acetic acid is excreta in urine in very large quantity. The enzyme monoamine oxidase convert serotonia to 5-HIAA. Reserpine drug used in the treatment of hypertension. It promotes action by monoamine oxidase and depresses cerebral function. The defect in the renal and intestinal transport of tryptophan leads to Hart up disease. The disease similar with pellagra. Large amounts of the amino acid are excreted in the urine and feces. Melatonin hormone is synthesized from serotonin.

Leucine is neutral essential ketogenic amino acid and forms an acetoacetate and acetate. It is branched chain amino acid and taken up by brain and muscle. In leucine metabolism, transamination gives α-keto isocaproic acid, which is converted into corresponding CoA, this is similar to oxidative decarboxylation of alfaketoglutarate and pyruvate. The enzyme complex is very important in the body of living organism. A deficiency of the enzyme causes maple syrup urine disease. In this disease the urine gives odor of maple syrup or burnt sugar, deterioration is rapid and results in mental retardation.

Valine is essential amino acid. It is widely distributed but rarely occurs in amount exceeding 10%. It is branched chain amino acid and can be derived from alanine by the introduction of two methyl group present on α - carbon atom. This is glycogenic. On deamination, it forms methyl-malonyl-CoA which can be converted to succinyl – CoA in place of two H atoms of the Methyl group.

Methionine is essential glycogenic amino acid. It is the only common amino acid possessing an ether linkage. Cereals have sufficient quantity of methionine whereas pulses lack in it. It is methylation product of homocysteine. Apart from its role as a protein constituent and as an essential aminoacid, methionine is also important as a donor of active methyl groups. Methionine is particularly important as a donor of methyl group in reaction known as transmethylation reactions. To act as a methyl donor, the methionine has to be first activated by ATP.

Phenylalanine is aromatic essential glucogenic and ketogenic amino acid. In metabolism phenylalanine is converted into tyrosine. In metabolism homogenstic acid is formed which undergoes cleavage and form fumarate and acetoacetate. The hormones such as adrenaline, noradrenaline, and thyrosine and melanin pigment formed from tyroxine. Several abnormalities observed in phenylanine metabolism such as phenylketonaria and alkaptonaria. In phenylketonaria, there is a black in hydroxylation of phenyl alanine to form tyrosine, this leads to mental retardation. Alkeptanaria, in this homogenstic acid is not further oxidised and excreted in urine. This lead to black urine.

Survey of literature[2] reveals that no work has been reported on complex tendencies of amino acids with transition metal ion copper (II) in ethanol-water solution. Therefore in order to understand the complex formation tendencies of amino acids with copper (II) in 80 % (v/v) ethanol-water medium at 27°C at a fixed ionic strength 0.1M NaCIO4

2. Materials and method

Amino acids in pure form were obtained from recognized chemical supplier and used as received. Ethanol was purified as described in literature.[3] Double distilled water was used for the preparation of ethanol-water mixture and stock solution of metal and amino acids.

All chemicals used were AnalaR grade. $NaClO_4$ (O.lM) and NaOH solution was prepared in carbon dioxide free double distilled water. Carbonate free NaOH was standardized by titrating with oxalic acid. $HClO_4$ Reidal (Germany) was used for the preparation of the stock solutions of copper (II) to prevent hydrolysis and standardized by using standard EDTA solution.

The experimental procedure, by the potentiometric titration technique, involves the titration of carbonate free solution of

1)Free $HClO_4$

2)Free $HClO_4$ + Ligand Amino acids

3)Free $HClO_4$ + Ligand Amino acids + Copper Metal Ion

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Against standard solution of sodium hydroxide and amino acid. The ionic strength of the solutions was maintained constant i.e. 0.1 M by adding appropriate amount of 1M sodium per chlorate solution. The titration were carried out at 27°C in an inert atmosphere by bubbling oxygen free nitrogen gas through an assembly containing the electrode to expel out $C0_2$. pH meter reading in 80%(v/v) ethanol-water were corrected by method of Vanuitert and Hass.[4] The formation constant of binary complexes were determined by computational programmed to minimize the standard derivation

3. Results and Discussion

Binary metal complexes

The proton ligand constant and metal ligand stability constant of amino acids with copper (II) determined in 80 %(v/v) ethanol-water mixture at 27°C and ionic strength μ= 0.1 M $NaCI0_4$ are given in Table I

According to Bjerrum and Martell et.al.[5] the formation of complex ML_N in general is a stepwise process and one has to deal with a series of equilibria of type

$M + L \rightleftharpoons ML$

$ML + L \rightleftharpoons IL_2$

$ML_2 + L \rightleftharpoons IL_3$

$ML_{(N-1)} + L \rightleftharpoons L_N$

The charge on the metal ion and ligand are omitted for brevity. The corresponding stepwise formation constants are then given by

$K_1 = a_{ML} / a_M \; a_L$

$K_2 = a_{ML2} / a_{ML} \; a_L$

$K_3 = a_{ML3} / a_{ML2} \; a_L$

$K_N = a_{MLN} / a_{ML(N-1)} \; a_L$

The stability constant (β_N) for overall equilibrium process,

$M + NL = ML_N$ is the product of the various stepwise formation constants and may be written as

$$\beta_N = K_1 . K_2 . K_3 \qquad K_N = \frac{{}^aML_N}{{}^aM \; {}^a(L)^N}$$

Writing in a similar fashion, for the ligand equilibria, the equations 1(a-d) can be written in the following form

$LH_{i-1} + H = LH_i$

Where LH_i is the ligand acid. The proton – ligand stability constant for such a reaction is given by

$$K^H_{i\,(T)} = \frac{{}^aLHi}{H_{i-1} \; {}^aH}$$

Where $K^H_{i\,(T)}$ is called the ith thermodynamic proton-ligand stability constant and is the reciprocal of the thermodynamic dissociation cons ⇆ of the acid LH_i dissociating as

$LH_1 \rightleftharpoons LH_{i-1} + H$

The pK_i value is given by

$$pKi = \frac{{}^aLH_{i-1} \; {}^aH}{{}^aLH_i}$$

For monobasic ligands $pK^H_1 = pK^H_i$ in magnitude.

For poly-basic acids $pK_1 = pK^H_n$

$pK^H_2 = pK_{n-1}$, $pK_n = pK_i$

The degree of formation or ligand number n is expressed as

$$\bar{n} = \frac{\sum_{i=0}^{N} i\beta_i(L)^i}{\sum_{i=0}^{N} \beta_i (L)^i} \qquad (\beta_o = 1)$$

A similar function for the proton-ligand complexes is given by

$$j_H$$

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$$\bar{n}_A = \frac{\sum_{i=0}^{H} i\beta_i (H)^i}{\sum_{i=0}^{H} \beta_i (H)^i} \quad (\beta_0 = 1)$$

Where $\bar{n}_A$ is the mean number of protons bound per noncomplex bound ligand molecule. The total concentration T_M of the metal M, is the sum of the concentrations of the different species containing it as

$$T_M = [M] + [ML] + \ldots + [ML_N]$$

$$N = \sum_{i=0} [Mli]$$

Similarly the total concentration of the ligand is the weighed sum of the concentrations of the species containing it as,

$$T_L = [L] + [ML] + 2[ML_2] + \ldots . + N[ML_N]$$

$$= [L] + \sum_{i=0}^{N} i]MLi]$$

The total concentrations T_M and T_L are given by the expressions.

$$T_M = [M] + \sum_{i=0}^{N} \beta_i [L]^I$$

$$T_L = [L] + [M] \sum_{i=1}^{N} i\beta_i [L[^i$$

The extent of the complex formation is characterised by the ligand number n, given as

$$\bar{n} = \frac{[ML[+ 2[ML_2] + \ldots . N[ML_N]}{[M] + [ML] + [ML_2] + \ldots . + [ML_N]}$$

$$\bar{n} = \frac{T_L - [L]}{T_M}$$

Where T_L is the concentration of ligand in all forms, (L) is the concentration of free chelating species and T_M is the total concentration of metal ion (bound or free). The ligand number 'n' is independent of concentration of both the metal ion and the ligand.

The determination of stability constants from the experimental data consist of three steps.

1) The construction of formation curve of the system. This is expressed as a plot on n against pL = log1/(L)
2) The calculation of K_1 values by solving the formation function of the system.
3) The conversion of the stoichiometric constants in to the thermodynamic functions.

The method of determinations of stability constants have described by Irving and Rossotti and Hear-on and Gilbert.[5] Irving and Rossotti made the use of potentiometric titration technique first used by Calvin and Melchior and now known as Calvin Bjerrum titration technique. The method of Irving & Rossotti has been employed in the present investigation.

The experimental procedure involves the titration of

i) Perchloric acid (A) ii) Perchloric acid + ligand (A+L)

iii) Perchloric acid + ligand + metal ion (A+L+M)

With standard solution of NaOH. The ionic strength of each solution is kept constant generally at 0.1 M by the addition of $NaClO_4$. The observed pH values are then plotted against the volume of alkali added. One thus obtains three titration curves, corresponding to the titration mentioned above. For the same volume of alkali, the ligand curve will indicate lower values of pH than the acid curve; it contains more titrable hydrogen ions, as it would happen when the chelating agent is an acid. If the metal chelate is formed in the reaction, the protons attached to the ligand must be displaced so that the metal complex titration curve will indicate pH values lower than the ligand titration curve.

The calculation n_A and $\bar{n}$ are made from the volumes of alkali required to obtain the same pH value in the ligand acid and metal ion titration. The displacement of the ligand curve

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from the acid curve and the metal curve, from the ligand curve is measured. The values of n_A (proton ligand formation number) for various pH values can be obtain from the equation

$$\bar{n}_A = \gamma - \frac{(V_2 - V_1)(N + E^0)}{(V^0 + V_1)(T_L^0)}$$

Where V^0 is the initial volume of the solution, E^0, T_L^0 are the total concentrations of the mineral acid and ligand respectively. V_1 & V_2 are the volumes of alkali of the known normality (N) required during the acid and ligand titration respectively at a particular pH. γ is the number of replaceable hydrogen ions.

Similarly, the metal – ligand formation number $\bar{n}$, can be calculated from the expression.

$$\bar{n} = \frac{(V_3 - V_2)[(N+E^0) + T^0_L(\gamma - \bar{n}_A)]}{(V^0 + V_2)\, n_A\, T^0_M}$$

Where V^0 and T^0_M represent the volume of alkali required obtaining the same pH as the ligand titration and the total concentration of the metal ion respectively. The other terms have the same significance as in the ligand titration

The free ligand concentration, pL can be calculated with the help of the following expression

$$pL = \log \left(\frac{\sum_{i=0}^{N} K_i [H]^{iH}}{T_L^0 - \bar{n} T_M^0} \times \frac{V^0 + V_3}{V^0} \right)$$

Or

$$pL = \text{Log} \left(\frac{1 + ([H^+]/K_1) + ([H^+]/K_1K_2) + \ldots}{T_L^0 - \bar{n} T_M^0} \times \frac{V^0 + V_3}{V_0} \right)$$

Above equation express n as a function of pL which is represented as the formation curve. The number of complexes formed in the reaction can be deduced from the formation curve and the values of stability constants can be determined.

The difference $(V_2 - V_1)$ was estimated from the graph accurately to second place of decimal and final values of n_A were obtained from the above equation.

a) Calculation of pK values:

i) Half – integral method: - The pK values of the ligands were initially calculated from the formation curves for polybasic acids, $pK_1 \ldots . pK_n$ corresponds to n_A values equal to (total number of displacement proton – 0.5) respectively.

ii) Method of point wise calculations:

The point wise calculations attempts to get pK's from various n_A, the expression used is

$$\log \frac{\bar{n}_A - (i-1)}{i - \bar{n}_A} = pK_i^H - pH$$

The values of $\bar{n}_A$ selected are in the range

$$\sum_{i=1} (i - 0.2) \quad \text{to} \sum_{i=1} (i - 0.8)$$

The following three methods were used to determine the stability constants.

a) Half-integral method:-

The values of $\log K_1$ and $\log K_2$ were calculated from the formation curve by the known value of pL at which n = 0.5 and 1.5 respectively.

b) Point wise calculation method:-

According to point wise calculation method, the metal ligand stability constants for 1:1 and 1:2 complexes were calculated by using the equation:

$$\log \frac{n - (\bar{N}-1)}{N - n} = \log K_N - pL$$

The range of n values are selected for the calculation are

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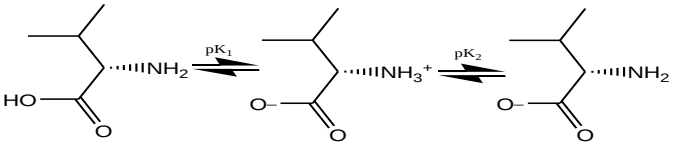
N=1 N=1
$\sum$ (N – 0.2) to $\sum$ (N – 0.8)
N=N N=N

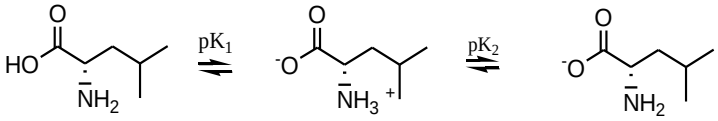
c) Least – Squares method:-

For the solution of formation function the methods suggested by Bjerrum and Irving and Rossotti [6] have been widely used. Bjerrum equates the half-integral values to appropriate log K values where K is a formation constant, as the first step in a series of successive approximation. This method has been criticised by Irving and Rossotti on the grounds that it uses only two point on the formation curve and it holds only when log (K_1/K_2) > 2.5, for system where only 1:1 and 1:2 complexes are formed. However, when a Calvin – Bjerrum pH titration technique is used to obtain the formation curve data, the logK values cannot be more accurate than observed pH values i.e. the accuracy cannot be greater than about + 0.02 log unit. In such cases half integral method give reasonably accurate values for even much lower value of log (K_1/K_2).

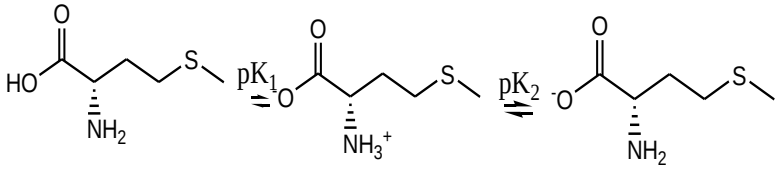
Proton ligand dissociation constant of amino acid is shown below.[7]



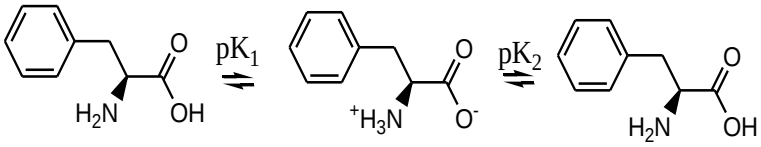
(Dissociation of Valine)



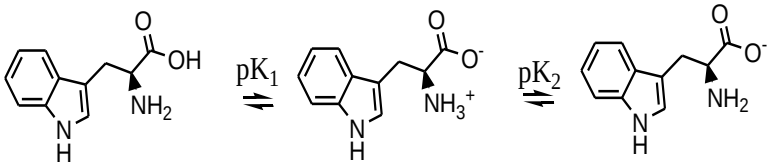
(Dissociation of Leucine)



(Dissociation of Methionine)



(Dissociation of Phenylalanine)



(Dissociation of tryptophan)

The proton ligand constant and metal ligand stability constant of dapsone and amino acids with copper (II) determined in 80%(v/v) ethanol-water mixture at 27^0C and ionic strength μ=0.1M $NaClO_4$ are given in Table. [8-14]

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Ligand	pK			
	pK_1	pK_2		
			Logβ	
Leucine	3.8100	10.3400	Log K_1	7.7078
			Log K_2	4.3500
Valine	3.2100	9.8024	Log K_1	5.6122
			Log K_2	3.5901
Methionine	3.1200	9.6000	Log K_1	3.1000
			Log K_2	-
Phenylalanine	3.1400	9.3000	Log K_1	6.4405
			Log K_2	5.3616
Tryptophan	3.8000	10.390	Log K_1	-
			Log K_2	-

4. Conclusion:

Proton ligand stability constant and metal ligand stability constant of amino acid with Cu(II) were determined at 27^0C and μ=0.1 m $NaClO_4$ in 80% (v/v) ethanol- water medium and presented in Table pK values of the ligands generally increase as the dielectric constant of the medium decreases thereby enhancing logK values. Similar trends are observed in the pK and logK values of amino acids and their complexes with Cu (II).

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