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Glycine and L-Tryptophan, a Comparative Investigation on Interactions in Cu(II) Binary and Ternary Complexes in Aqueous Solution

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Abstract The acidity and stability constants of M(Trp)[i] M: Cu^{2+}, $Cu(Bpy^{ii})^{2+}$, and $Cu(Phen^{iii})^{2+}$ complexes, were determined by potentiometric pH titration. It is shown that the stability of the binary Cu(Trp) complex is determined by the basicity of the carboxylate group on one side and amino group on the other side. It is demonstrated that the equilibrium, $Cu(Har^{iv})^{2+} + Cu(Trp) \rightleftharpoons Cu(Har)(Trp) + Cu^{2+}$, is displacement due to the well known experience that mixed ligand complexes formed by a divalent 3d ion, a heteroaromatic N base and an O donor ligand possess increased stability. The other part of this displacement, which amount on average to an increased stability of the mixed ligand Cu(Bpy)(Trp) and Cu(Phen)(Trp) complexes of about 0.97 or 1.31 log unit. The stability constants of the 1:1 complexes formed between Cu^{2+}, $Cu(Bpy)^{2+}$ or $Cu(Phen)^{2+}$ and Trp^{2-}, were determined by potentiometric pH titration in aqueous solution (I = 0.1 M, $NaNO_3$, 25℃). The order of the stability constants was reported. The results show following order for Trp, Cu(Trp) < Cu(Bpy)(Trp) < Cu(Phen)(Trp), and Gly, Cu(Gly) > Cu(Bpy)(Gly) ≤ Cu(Phen)(Gly). A comparative investigation between ternary complexes of Trp and Gly[v] is made. The comparison of stability constants of these ternary complexes show that Cu(Har)(Gly) exist in open form but Cu(Har)(Trp) is found near 100% in closed form. The differences between the above mentioned stability constants based on stacked form of Cu(Har)(Trp). The stacked form provides for increased stability.

Keywords Glycine**,** Tryptophan, Divalent Metal Ions, Potentiometric Titration, Acidity and Stability Constants

1. Introduction

L-Trp or D-Trp; sold for medical use as Tryptan (fig. 1)[1] is one of the 20 standard amino acids and essential in the human diet. It is encoded in the standard genetic code as the codon UGG. Tryptophan (Trp) is considered exceptional in its diversity of biological functions[2]. It is a vital constituent of proteins and indispensable in human nutrition for establishing and maintaining a positive nitrogen balance[3]. Besides, some of its derivatives are potent drugs[4]. Trp is widely used in food industry. It is sometimes added to dietary and feed products as a food fortifier in order to maintain the amino acid balance of the food and correct possible dietary deficiencies. Trp can also be used to study structure and dynamics of the proteins because of its indole moiety[5]. In particular, Trp is the precursor of the neurotransmitter serotonin and plays an important role in brain function and related regulatory mechanisms[6]. In addition, Trp is an important and frequently used starting material in the chemical synthesis of a range of pharmaceuticals[7].

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The importance of noncovalent interactions for the shape of macromolecules, the selectivity in biological system is generally accepted and especially hydrophobic and stacking interactions, which have been considered in mixed ligand complexes[8-10]

The distinguishing structural characteristic of tryptophan is that it contains an indole functional group. It is an essential amino acid as demonstrated by its growth effects on rats. Now it is interesting to investigate the complex bilding of ternary systems with Trp. We would like to determine the thermodynamic constants of ternary complexes such as Cu(Har)(Trp). This kind of structure of Trp complex can show new aspect of Trp's properties in biological systems.

2. Experimental

2.1. Materials

Chemicals were purchased from Merck. L-tryptophan, copper(II) nitrate trihydrated, sodium nitrate, potassium hydrogen phthalate and standard solutions of sodium hydroxide (titrasol), 2,2'-bipyridyl, 1,10-phenanthroline, nitric acid, EDTA and of the buffer solutions of pH 4.0, 7.0 and 9.0 were from Merck. All the starting materials were pro analysis and used without further purification. Water was purified

by Mili-Q water purification system, deionized and distillated.

2.2. pH Titrations

Reagents: Carbonate-free sodium hydroxide 0.03 M was prepared and standardized against sodium hydrogen phthalate and a standard solution of nitric acid 0.5 mM. Copper (II) nitrate solution (0.03 M) was prepared by dissolving the above substance in water and was standardized with standard solution of EDTA 0.1 M (triplex).

2.3. Apparatus

All pH titrations was performed using a Metrohm 794 basic automatic titrator (Titrino), coupled with a Hero thermostating bath at 25℃ (±0.1℃) and a Metrohm combined glass electrode (Ag/AgCl). The pH meter was calibrated with Merck standard buffer solutions (4.0, 7.0 and 9.0).

2.4. Procedure

For the determination of acid dissociation constants of the ligand Trp an aqueous solution (0.3 mM) of the protonated ligand was titrated with 0.03 M NaOH at 25℃ under nitrogen atmosphere and ionic strength of 0.1 M, $NaNO_3$. For the determination of binary (one ligand and Cu^{2+}) and ternary systems (Cu^{2+}, one of the other L ligand (Har) and Trp), the ratios used were 1:1:1, Cu(II): Trp : Har, 0.3 mM. This solution was titrated with 0.03 M NaOH under the same conditions mentioned above. Each titration was repeated seven times in order to check the reproducibility of the data.

Calculation

The acid dissociation constants, $K^{H}_{H_2(Trp)}$ and $K^{H}_{H(Trp)}$ for H_2(Trp) were calculated by an algebraic method. The equilibria involved in the formation of 1:1 complex of Trp and a divalent metal ion may be expressed as equations (3) & (4).

3. Results and Discussion

Table 1. Logarithm of the stability constants of binary and ternary complexes of M^{2+} at 25℃, 0.1 M, $NaNO_3$*

$pK^{H}_{H_2(Trp)} = 2.22\pm0.08$			$pK^{H}_{H(Trp)} = 9.14\pm0.03$	
$pK^{H}_{H_2(Gly)} = 2.49\pm0.08$			$pK^{H}_{H(Gly)} = 9.36\pm0.03$	
No.	Species	logKa	Δlog Kb	Ref.
1	Cu(Trp)	8.05±0.05	-	[13]
2	Cu(Bpy)(Trp)	9.02±0.06	0.97±0.08	-
3	Cu(Phen)(Trp)	9.36±0.08	1.31±0.09	-
4	Cu(Gly)	7.06±0.08		[18]
5	Cu(Bpy)(Gly)	5.95±0.08	−1.11±0.11	[18]
6	Cu(Phen)(Gly)	6.12±0.07	−0.94±0.11	[18]

*The given errors are three times the standard error of the mean value or the sum of the propabable systematic errors, [a]according eq. (4), [b]according eq. (8).

3.1. Acidity Constants

Tryptophan (Trp) can accept one proton on carboxylic group, for which the following deprotonation equilibria hold:

$$H_2(Trp) \rightleftharpoons H^+ + H(Trp)^- \quad (1a)$$

$$K^{H}_{H_2(Trp)} = [H(Trp)^-][H^+]/[H_2(Trp)]$$

Trp can release one other proton from amine group according following deprotonation equilibria (tab.1, see sec.2):

$$H(Trp)^- \rightleftharpoons H^+ + Trp^{2-} \quad (2a)$$

$$K^{H}_{H(Trp)} = [Trp^{2-}][H^+]/[H(Trp)^-] \quad (2b)$$

Also the two protons in H_2(Trp) are certainly bound at the terminal acetate group and amine group, i.e., it is released from $-CO_2H$ or $-NH_2$ according to equilibrium (1) & (2). These values are, as accepted, close to the pKa values of $-CO_2H$ which is 2.22[8].

3.2. Stability of Binary and Ternary Complexes

If we abbreviate for simplicity Cu^{2+}, $Cu(Bpy)^{2+}$, and $Cu(Phen)^{2+}$ with M^{2+}, one may write the following two equilibria (3) & (4):

$$M^{2+} + H(Trp)^- \rightleftharpoons M(H;Trp)^+ \quad (3a)$$

$$K^{M}_{M(H;Trp)} = [M(H;Trp)^+]/[M^{2+}][H(Trp)^-] \quad (3b)$$

$$M^{2+} + (Trp)^{2-} \rightleftharpoons M(Trp) \quad (4a)$$

$$K^{M}_{M(Trp)} = [M(Trp)]/[M^{2+}][Trp^{2-}] \quad (4b)$$

The experimental data of the potentiometric pH titrations may be completely by considering the above mentioned equilibria (1) through (4), if the evaluation is not carried into the pH range where hyrdoxo complex formation occurs.

The stability of ternary complexes may be evaluated by the following equilibrium:

$$mA + nB + qM + rH \rightleftharpoons A_mB_nM_qH_r \quad (5a)$$

where M is the metal ion, H is the proton, A and B are the ligands. The global stability constants for the ternary complexes may be represented as following:

$$\log\beta_{pqrs} = [A_mB_nM_qH_r]/[A]^m[B]^n[M]^q[H]^r \quad (5b)$$

It is possible to define the stability constants for ternary complexes in relation to their binary ones[9], represented by the equilibrium (6) & (7).

$$M + L_1 \rightleftharpoons ML_1 \quad (6a)$$

$$K^{M}_{M(L_1)} = [ML_1]/[M^{2+}][L_1] \quad (6b)$$

$$ML_1 + L_2 \rightleftharpoons ML_1L_2 \quad (7a)$$

$$K^{M}_{M(L_1L_2)} = [ML_1L_2]/[ML_1][L_2] \quad (7b)$$

Differences between the stability constants of the ternary and binary complexes show the tendency of the formation of ternary species[10]. This could be expected by Eq. (8):

$$\Delta\log K = \log K^{ML_1}_{M(L_1L_2)} - \log K^{M}_{M(L_2)} = \log K^{ML_2}_{M(L_1L_2)} - \log K^{M}_{M(L_1)} \quad (8)$$

The difference between the constant refined from experimental data and those calculated statistically using Eq. (8) indicates the possibility of ligand-ligand interaction.

3.3. Potentiometric Analyses

The model of species for these ternary systems that was used in superquad program includes all the species of table 1 as well as the hydrolysis of Cu^{2+}[11,12]. The stability constants of the binary complexes were refined separately using the titration data of this system in a 1:1 and 1:2 ligand: Cu^{2+} ratio in the same conditions of temperature and ionic strength.

They were fixed and, consequently, only ternary species were refined in ternary model of the species. The results are summarized in Table 1. The order of the resulted stability constants are Cu^{2+} < $Cu(Bpy)^{2+}$ < $Cu(Phen)^{2+}$. Figure 2 shows schematic structures of the species with interactions according to equilibrium (4) & (7) for Cu(Phen)(Trp). The results of the acidity constants show good agreement with reported values[13]. The reported stability constant of Cu(Trp) complex is similar to our results (tab. 1).The difference between stability constants according eq. (8) show that mixed ligand complexes[14-17] formed by a divalent 3d ion, a heteroaromatic N base and an O donor ligand possess increased stability. Now one can calculate the free energy ΔG, used ΔlogK received from eq. 8 (tab. 1). We receive for $\Delta\log K^{Cu(Bpy)}_{Cu(Bpy)(Trp)}$ 5.44 kJ/mol and for $\Delta\log K^{Cu(Phen)}_{Cu(Phen)(Trp)}$ 7.34 kJ/mol, which are considerable high. This means that interaction between $Cu(Har)^{2+}$ and trp^{2-} is relative strong and the observed increased stability indicate strong complex bilding of ternary systems.

It has to be further emphasized that the basicity of the carboxylate group in aqueous solution is very low and consequently this also applies for the coordinating properties of this group.

Comparison of the stability constants for the Cu(Bpy)(Trp) and Cu(Phen)(Trp) complexes in table 1 with the corresponding values for Cu(Trp) indicates in increased stability of the mixed-ligand species. As it is well known for a number of Cu(Her)(L) complexes that an increased complex stability is connected with the formation of intramolecular stack between the aromatic ring systems of 2,2'-Bipyridyl and 1,10-phenanthroline and the heteroaromatic ring of Trp (opened form ↔ closed form)[10]. The difference, if it exist, between these last mentioned constants and the experimentally ligand-ligand stack interaction in the Cu(Har)(Trp) complexes.

Table 2. Extent of intramolecular stack formation in ternary Cu(Har)(L) complexes as calculated from stability constants (eq. 7). Intramolecular and dimensionless equilibrium constant K_I (eq. 9) and percentage of stacked $Cu(Har)(L)_{cl}$ species in aqueous solution at 25°C, 0.1 M, $NaNO_3$*

No.	Species[a]	Δlog K[b]	ΔΔlog K[c]	K_I[d]	%$Cu(Har)(L)_{cl}$[e]
1	Cu(Bpy)(Trp)	0.97±0.08	2.08±0.14	119.23±38.76	99.17±0.27
2	Cu(Phen)(Trp)	1.31±0.09	2.25±0.14	176.83±57.34	99.44±0.18
3	Cu(Bpy)(Gly)	–1.11±0.11	–	–	–
4	Cu(Phen)(Gly)	–0.94±0.11	–	–	–

*The given errors are three times the standard error of the mean value or the sum of the propabable systematic errors. [a]from table 1, [b]according eq. (8), [c]according eq. (9), [d]according eq. (11) , [e]according eq. (12).

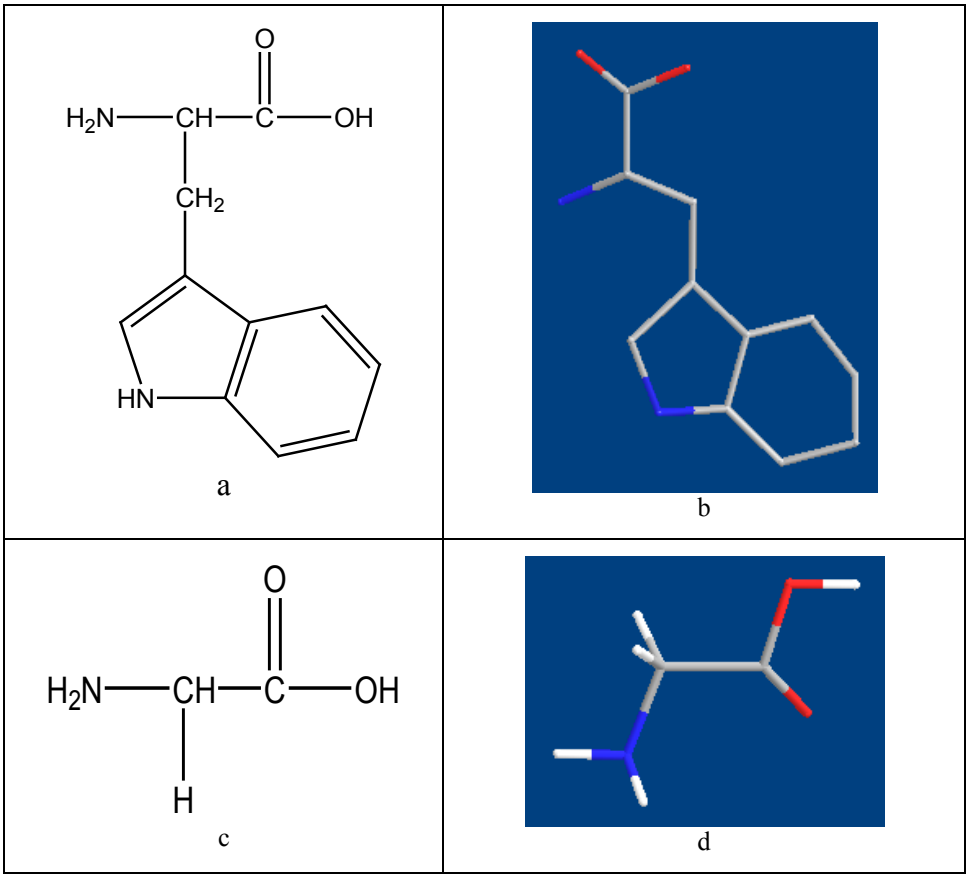

Figure 1. Chemical formula of L-Tryptophan (a,b), Glycine (c,d).

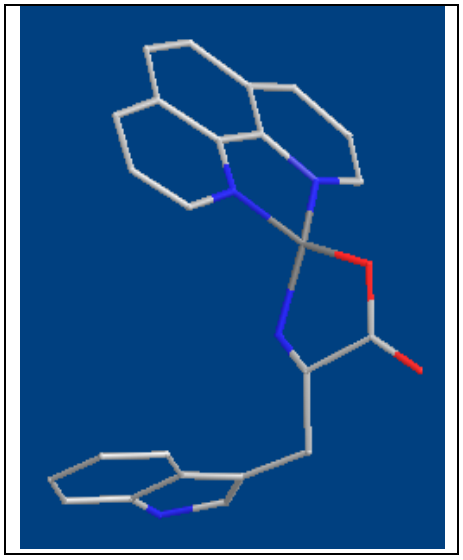

Figure 2. Schematic structures of the species with interactions according to equilibrium (4) & (7) for Cu(Phen)(Trp). The structure in the right part of the figure was drawn with the program CS Chem 3D, version 3.5, from Cambridge Software Corporation

As we can see from the experimentally results from table 1, there is no increased stability constants in case of Cu(Har)(Gly), this means that there is no indication of intramolecular stack interactions. For this reason we can use the stability constants of Cu(Har)(Gly) as opened form in our next calculations.

By employing eq. (8) the following definition is possible (eq. (9)):

$$\Delta\Delta\log K = \Delta\log K_{cl} - \Delta\log K_{op} \quad (9)$$
$$= \Delta\log K_{Cu(Phen)(Trp)} - \Delta\log K_{Cu(Phen)(Gly)}$$

It is evident that the coordination sphere of Cu^{2+} ions on both sides of this equilibrium are identical, consequently the value for $\Delta\Delta\log K$ is a true reflection of the extent of the intramolecular hydrophobic or stacking interaction in Cu(Har)(Trp) complexes. The corresponding results are listed in the fourth column of table 2.

Now we can define the intramolecular and thus dimensionless equilibrium constant K_I is than given by equation (10) for opened and closed form:

$$K_I = [Cu(Phen)(Trp)]_{cl} / [Cu(Phen)(Trp)]_{op} \quad (10)$$

The observed increased complex stability is linked to K_I by equation (11):

$$K_I = 10^{\Delta\Delta\log K} - 1 \quad (11)$$

Knowledge of K_I allows calculation of percentage of the macrochelated form according to equation (12)[10]:

$$\% \text{ Cu(Har)(Trp)} = 100 * K_I/(1+K_I) \quad (12)$$

The results of the calculations of above mentioned equations are summarized in table 2.

Comparison of the percentage of the macrochelated form according to equation (12) in the table 2 shows the high stacking tendency of Trp based on heteroaromatic structure of indole moiety[5].

The distinguishing structural characteristic of tryptophan is that it contains an indole functional group. It is an essential amino acid as demonstrated by its growth effects on rats. Now it is interesting to investigate the complex bilding of ternary systems with Trp. The comparison of stability constants of these ternary complexes show that Cu(Har)(Gly) exist in open form but Cu(Har)(Trp) is found near 100% in closed form (see last column in tab. 2). The differences between the stability constants are based on stacked form of Cu(Har)(Trp). The last provides for increased stability. The results described in this study show that Trp is a very versatile ligand. Due to the dominating conformation in aqueous solution hardly any macrochelates are formed in Cu(Har)(Trp) complexes. The energy differences between closed and open form in Cu(Har)(Trp) is significant. One can calculate the free energy ΔG for Cu(Har)(Trp). So we receive respectively values for Cu(Bpy)(Trp) and Cu(Phen)(Trp) 11.66 kJ/mol and 12.62 kJ/mol. The according structure of ternary Cu(Phen)(Trp) is shown in figure 2.

Due to the resulting data is very interesting that affects the ternary complexes of Trp in biological systems as active. This might be used, for example in the case of cell separation. The inhibition of DNA cleavage and block the cell divisions can be influenced by strong stack bilding of Har and Trp with nucleotide bases [18-21].

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[i] Trp: L-Tryptophan
[ii] Bpy: 2,2'-Bipyridyl
[iii] Phen: 1,10-phenanthroline
[iv] Har: Heteroaromatic ligand such as Bpy or Phen
[v] Gly: Glycine