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Potentiometric study of binary complexe of amino acid glycine with metal ion Zn^{2+} In aqueous solution

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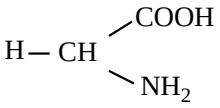
Abstract
The protonation and stability constant of complexation between glycine amino acid with Zn^{2+} ion in Aqueous Solution were studied potentiometrically. All of titration procedures were carried out at 25±0.1 ^{0}C and ionic strength (μ) of 0.1 M supported by $NaNO_3$. The protonation and overall stability constants (logβ's) of species has been evaluated by computer refinement of pH-volume data using BEST computer program. Several model are tested and based on the lowest σ_{FIT}, the best one is accepted. The distribution diagram of all species in absence and presence of metal ion as function of pH with SP and SPE programs was given.

Keywords: Potentiometric Study, Stability Constant, glycine (Gly), BEST Program.

Introduction
Many transition and heavy metal cation play an active role in a great number of biological processes, being components of several vitamins and drugs. We have been interested in studying the interaction of Zn^{2+} metal ion with amino acid glycine, because Zinc is an essential micronutrient required for many cellular processes, especially for the normal development and function of the immune system. Zinc homeostasis and signaling are critical in immune activation, and an imbalance in zinc homeostasis is associated with the development of chronic diseases. Zinc deficiency causes significant impairment in both adaptive and innate immune responses, and promotes systemic inflammation [1]. Zinc (Zn) nutritional importance has been known for a long time, but in the last decades its importance in immune modulation has arisen. Zn is a ubiquitous element in cells, present in the cytoplasm and in 80 most organelles. Zn functions as a key structural or catalytic component of more than 300 enzymes and it is implicated at all levels of cellular signal transduction. In this way, Zn regulates also cell–cell communication, cell proliferation differentiation and survival [2]. Zinc Glycine amino acid chelate is a superior bioavailability and had a high-potency source of zinc formulated for enhanced absorption. In this form, zinc is coupled with two glycine molecules to facilitate its absorption across the intestinal wall and reduce interference from phytates and competing minerals [3]. Therefore in the present work, binary system of Zn2+and glycine in mentioned conditions was studied and its stability constant has been obtained. These binary systems were fined as new species and also its stability constants have not been reported until now. There are noticeable points that stability and protonation constants of some species are in good agreement with reported species. Potentiometric titration is accepted as a powerful and simple electroanalytical technique to determine protonation and stability constants. It is well known that proton transfer plays an important role in the reaction in aqueous solution such as complex formation acid and base catalyses and enzymatic reaction [4]. Thus, the accurate determination of acidity and stability constants values are fundamental to understanding the behavior of amino acid and their interaction with metal ion in aqueous solution. The simplest electroanalytical technique for determination of stability constants is potentiometric titration system using glass electrode. The stability constants can be of significance in order to predict different chemical processes such as isolation, extraction, or

preconcentration methods [5,6].The purpose of this work is to investigate the protonation constants of Glycine (Figure 1) and stability constants of complex formation with metal ion Zn^{2}+ (Figure 2) using the BEST program developed by Martell and his coworkers [7] for potentiometric titration.



Scheme 1. Chemical structure of Glycine (Gly)

Glycine (aminoethanoic acid), is the simplest amino acid, its side chain consists of just a single hydrogen atom. Because of its simplicity, it has only one form, not two (l- or d-) like other amino acids. Glycine has been shown to support healthy kidney and liver function as well as nervous system health. In animal studies and in a small human study, glycine demonstrated the potential to support memory and mental function [8]. Glycine is an amino acid that can exist in aqueous solution in three different forms, namely $^{+}H_3NCH_2COOH$ (cation), $^{+}H_3NCH_2COO^{-}$ (zwitterion) and $H_2NCH_2COO^{-}$ (anion) (Eq.1).

$$\underset{GlyH_2^+}{H_3N^+CH_2CO_2H} \rightleftharpoons \underset{zwitterion(GlyH)}{H_3N^+CH_2CO_2^-} \rightleftharpoons \underset{Gly^-}{H_2NCH_2CO_2^-} \qquad (1)$$

2. Experimental

2.1. Apparatus and materials

All potentiometric pH measurements were made using a Model 686 Metrohm Titoprocessor equipped with a combined glass-calomel electrode.All common laboratory chemicals used were of reagent grade from Merck (Darmstadt, Germany). Analytical grade nitrate salts of metal ion and amino acid Glycine with the highest purity available are purchased from Merck Company and used without any further purification. Carbonate free NaOH solution was standardized with potassium hydrogen phethalate. The HNO_3 solution was standardized with standard NaOH. All solutions were prepared in doubly distilled deionized water.

2.2. Potentiometric Measurments

The procedures employed for the potentiometric pH measurements have been described in detail [9]. In general, an experimental run involves collecting equilibrium data points throughout the entire pH range, between 2.0 and 11.50 as a function of millimoles standard NaOH, added using the piston buret through a fine capillary tip immersed in the solution. In titration, after each addition, the required time was allowed to reach chemical equilibrium.

All potentiometric pH measurements were done on solution in a 75-mL double-walled glass vessel using a Model 686 Metrohm Titoprocessor equipped with a combined glass-calomel electrode and the temperature was controlled at 25.0 ± 0.1 °C by circulating water through the double-walled glass vessel, from a constant-temperature bath (home made thermostat). The cell was armed with a magnetic stirrer and a tightly fitting cap, through which the electrode system and a 10-mL capacity piston burette were inserted and sealed with clamps and O-rings. Atmospheric CO_2 was excluded from the titration cell with a purging steam of purified nitrogen gas. The system was maintained at an ionic strength of μ=0.10 M with $NaNO_3$ as a supporting electrolyte.

Stability constant of complexe with metal ion Zn^{2+} was carried out according to the literature [7,10-12]. The required amount of $NaNO_3$ (from a 0.50M stock solution), HNO_3 (0.10 M) and 0.0125 M of Zn^{2+} metal ion and 0.02 M Glycine and doubly distilled deionized water were added. To the required amount of doubly distilled

deionized water was added to the cell to a total volume of 40 mL. The potentiometric study operates at the metal: amino acid molar ratios of 1:2. The accuracy rapidly decreases for higher molar ratios due to the fact that the characters of the titration curve of the complexes and the titration curve of the amino acid without metal ion are similar. The mutual ratios cannot be used because of the metal hydrolysis. Solution was titrated potentiometrically with a CO_2 –free 0.09860 M solution NaOH.

In the systems studied, the titration was performed up to pH values at which the formation of precipitates began and unstable emf measurements were obtained. From the titration curve of the first solution the acidic protonation constant of the amino acid and from the second titration curve the formation constants of different form of complexes was evaluated using the BEST program described by Martell and Motekaitis [7] The value of $K_w = [H^+][OH^-]$ used in the calculation was $10^{-13.78}$ [13].

The protonation and formation constants of all species were obtained through the least-squares refinement of its $p[H^+]$ profiles. Throughout this investigation the function minimized was the weighted average of the sums of squares of deviation between calculated and observed $p[H^+]$ value (σ_{fit}).

3. Results and Discussion

The carboxylate group is an important class of amino acid in inorganic and bioinorganic chemistry. Metal complexes containing monocarboxylic acids are well known and the publication of many structurally characterized examples of this class of compound has demonstrated the versatility of the carboxylate group as an inner sphere amino acid [14].The amino acids have special importance among the other chemical groups because they are the foundation stones of living organisms. The determination of the microscopic protonation constants is of great importance for the elucidation of numerous biological compounds. In addition, the microscopic protonation constants of various amino acids investigated in this study in water containing media.

The standard α-amino acids have special importance among the other chemical groups since they are found in all naturally occurring proteins, which play a vital role in nearly all chemical and biological processes. Despite their recognized importance, there are only a few experimental contribution on their acid–base behavior in different environments [15].

3.1. Protonation Constants

The protonation constants of Glycine were obtained under the same condition of ionic strength and temperature which are applied for the study of binary systems. The overall and stepwise protonation constants of studied amino acid were calculated from computer refinement of pH–volume data using best program and presented in Table 1.

Table 1. Deprotonation constants of Gly studied at 25± 0.1 °C in aqueous KNO_3 (μ = 0.100 M).

Amino acid	H	L	Logβ	Pk_a
Gly	1	1	9.71	9.71
	2	1	12.69	2.98

We remark that are in good agreement with previously reported data [7,10-12]. The small differences are within the limits of experimental errors or due to difference in experimental condition (i.e. different in ionic strength and temperature). The maximum number of protons attached to glycine 2. Deprotonation constants calculated for glycine are 2.98 ; 9.71 as to carboxylate groups at acidic pH and amine groups at basic pH.

The species distribution curves of Glycinehave been shown in Figure 1 .As can be seen from the species distribution diagram, two groups on each amino acid protonated at pH ≈3, respective protonated form is LH_2. When pH is increased, the amino acids lose gradually the protons and convert to the other various forms, LH and L. The formation of free amino acid (L) starts at pH 6.80 for Glycine and reaches maximum concentration at pH ≈11.50.

The deprotonation equilibrium is as seen in the following equation(K_n ; n=2) Eq (2): (amino acid charges are omitted for simplicity).

$$LH_n \leftrightarrow LH_{n-1} + H^+ \qquad K_n = [LH_{n-1}][H^+]/[LH_n] \qquad (2)$$

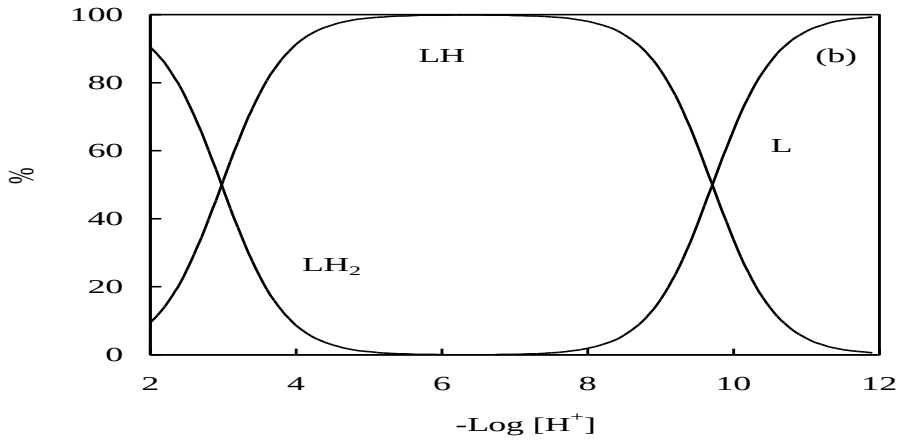


Figure 1: Distribution curves of Gly species

3.2. Binary Complex Formation Equilibria

The formation constants of Zn^{2+} with Glycine as binary complexes were determined under condition identical .To determine the stoichiometry and stability constants of desired complex, which take place between the amino acid and metal ion, the solution including metal ion (M), the amino acid (L) and fixed amount of nitrate potassium have been titrated with standard NaOH solution. The obtained values by the BEST program for the compounds are given in Table 2. They show good agreement with the reported values [7,10-12] after allowing for change in experimental condition as well as in method of calculation. The cumulative stability constant (ßmlh) are defined by equation (3) (chargeis omitted for simplicity).

$$mM + 1L + hH \rightleftarrows M_mL_lH_h \quad ß_{mlh} = [M_mL_lH_h]/[M]^m[L]^l[H]^h \qquad (3)$$

Where M is metal ion, L is Amino acid and H is proton and m, l and h are the respective stoichiometric coefficients. Since the amino acid and complexe activity coefficients are unknown, the $ß_{mlh}$ values are defined in terms of concentration. In the evaluation of the three component experimental data, the binary complex model was consider as known. The errors are minimized by use of a high constant ionic strength of 0.1 M $NaNO_3$ and amino acid concentration of 0.02M. The titration curves are given in Fig.2. As seen in Figure 2, the divergence between the titration curves, it can be concluded that strong interaction exists between metal ion and Glycine, in aqueous solution. The experimental curve was used to calculate the equilibrium constants for the reaction between the metal ion and amino acid. It should be noted that the stability constants result from a balance between the binding energy and the 3131witterio energies of all charge species involved in complexation reaction [16].

Although the experimental conditions are similar, their end point is different from each other because of the various degree of hydrolysis of the metal ion. When the hydrolysis degree of M is increased, the end point of the complex system shifts to right. The interaction of M with L (1:2) leads to form ML_2 type complex. The data obtained from metal ion complex with Glycine titration have been evaluated using BEST program and the species distribution curve was obtained from calculation.

3.3 Zn^{2+}-Q Binary Systems (Q=Gly)

For stability constants of the complex formation of amino acids with metal ion, the dissociation constant of amino acids are used. It is known that the reaction of peptides, proteins, and enzymes with metal ion are of biochemical importance. The explanation of these phenomena in the biological systems is possible only by determining the protonation constants of the amino acids as well as their stability constants, which are the measure of their tendency to make complexes with other metal ion.

Glycine appears in the anionic form over a much wider pH range when complexing metal ion than when in the free form free glycine appears principally in the zwitterionic form between pH 2.35 and 9.77. The anion only predominates above pH 9.77. The zwitterionic form of glycine is clearly less able to form stable metal ion complexe. 3132witterions can only coordinate through its carboxylate group, whereas the anion can give bidentate chelation through both the carboxylate and the amine groups [17]. Glycineis the simplest amino acid and very important amino acid present in collagen and synthesized from serine . Serine is involved in the synthesis of sphingol and therefore in the formation of sphingomyelins (phospholipids) in the brain [18].

In the Zn^{2+}-Gly system the species ZnQ, ZnQH, ZnQ_2, ZnQ_2H, ZnQ_2H_2, ZnQOH, $ZnQ(OH)_2$, ZnQ_2OH and $ZnQ_2(OH)_2$ are remained in the final refined model. The resulting formation constants of species obtained are listed in Table 2 and species distribution diagram are displayed in Fig. 2.

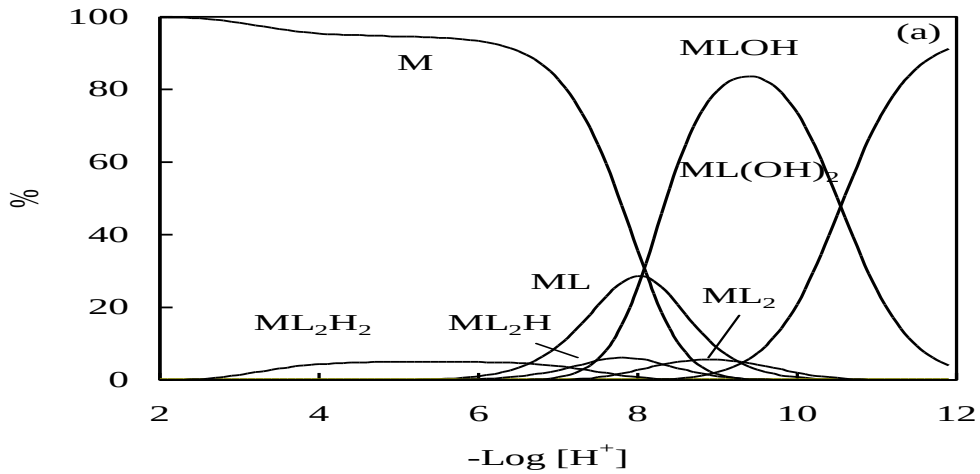


Figure 2: Species distribution curves of binary systems of Zn^{2+} - Gly at 25± 0.1 °C and ionic strength 0.1 M KNO_3.

Table 2: Overall stability constants for the interaction of H^+ (h) and Zn^{2+}(m) with Gly (l) their 1:2 mixture at 25± 0.1 °C and ionic strength 0.1 M KNO_3.

System	m	l	h	Logβ		
				P.W	Ref.	
Zn^{2+}-Gly	1	1	0	4.84	4.85	[19]
	1	1	2	16.07		
	1	2	0	8.95		
	1	2	1	17.88		
	1	2	2	25.81		
	1	1	-1	-2.97		
	1	1	-2	-15.51		
	1	2	-1	-3.12		
	1	2	-2	-12.65		

Conclusion

In the present paper protonation constants of the amino acid and formation constants of the resulting complexes were computed from pH-volume titration data using BEST program. Several models were tested and the best one, which regard to the least sum-of-squared deviation between pH_{cal} and pH_{obs}, was chosen. The computer refinement of the titration data showed the presence of ZnQ, ZnQH, ZnQ_2, ZnQ_2H, ZnQ_2H_2, ZnQOH, $ZnQ(OH)_2$, ZnQ_2OH and $ZnQ_2(OH)_2$species in binary system .The distribution diagram of detectable species in absence and presence of metal ion as function of pH was given. The overall protonation constants of amino acid glycine were calculated from computer refinement of the pH–volume data and the results are presented in Tables 1–2 and Figs. 1–2. Actually, there are competition between protons and metal ion for the complex formation due to interaction with donor groups in the amino acid.

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