Determination of Stability Constants of Mixed Ligand Complexes of Sulfanilamide and Other Bioactive Ligands with Cu(II) by Potentiometric Titration Method

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Received: 7 May 2013;

Accepted: 25 June 2013;

Published online: 28 April 2014;

AJC-15074

The stability constants of ternary complexes of Cu(II) with sulfanilamide drug (S) as primary ligand and some of bioligands viz., amino acids and peptides as secondary ligand were determined pH-metrically at 25 °C and ionic strength 0.1 mol /L NaNO₃ in aqueous solution. The stability of each ternary complexes was investigated and compared with those of the corresponding binary complexes in terms of the $\Delta \log K$ parameter. The concentration distribution of the complexes formed in solution was evaluated.

Keywords: Potentiometric studies, Sulfanilamide, Mixed ligand complexes, Stability constants.

INTRODUCTION

It is well known that the medicinal inorganic chemistry is a multidisciplinary field combining elements of chemistry, pharmacology, biochemistry and medicinal chemistry¹. Most drugs offer a range of potential donors for metal ions, including carboxylate, amine, thiolate, phosphate and aromatic nitrogen groups². It can exploit the unique properties of metal ions for the design of new drugs³. Over the last few years was found that the complexes of drugs has higher efficacy than parent drugs. For example, copper salicylate has a better antiinflammatory effect than cortisones with lesser undesired effects and also possesses good anticancer and anticonvulsant activity⁴. Beside, it was found that copper complexes of all antiepileptic drugs show better efficacy and less toxicity than the parent drugs⁵.

Further more, the stability constant of metal complexes with drugs are useful to know the proper dose of drug and their effect with all other components of blood stream as well as to measure the strength of metal ligand bonds⁶⁻⁹. A polarographic technique was used to determine the stability constants of Mn²⁺ complexes with sulfanilamide drug and cephalirin at pH = 7.30 ± 0.01, confirming that either sulfanilamide or cephalothin or its complexes could be effective against Mn²⁺ toxicity¹⁰. On the other hand, sulfanilamide (S) is one of the most common antimicrobials and the parent compound of a group of sulfa drugs¹¹. Chemically, it is a molecule containing the sulfonamide functional group attached to an aniline (Fig. 1), it is used in treatment of meningitis, tonsillitis, pneumonia,

gonorrhea and sinus infections^{11,12}. In the present study, the results of potentiometric study on the protonation constants, stability constants of binary and ternary systems of copper(II) ion with sulfanilamide and the amino acids or peptides are reported in aqueous solution at 25 °C and ionic strength 0.1 M NaNO₃.

Fig. 1. Structures of sulfanilamide drug

EXPERIMENTAL

Sulfanilamide (S) was obtained from BDH. The amino acids glycine, alanine, valine, proline, β -phenylalanine, methionine, isoleucine, glutamic acid, threonine, serine, ornithine, cysteine and histidine, together with histamine - 2 HCl, penicillamine, imidazole, mercaptoethanol and methylamine, were provided by Sigma. The peptides used are glycylglycine, glycineamide and glutamine, also provided by BDH-Biochemicals Ltd. Threonine, serine, ornithine, cysteine, histidine and penicillamine solutions were prepared in the protonated form by dissolution in two equivalents of nitric acid. Stock solutions of NaNO₃ and HNO₃ (Merck) were prepared in deionized water. Cu(NO₃)₂·6H₂O and NaNO₃ were provided by BDH-Biochemicals Ltd. The copper content of solutions was determined by complexometric EDTA titrations¹³. Carbonate-free

NaOH (titrant) was prepared and standardized against a potassium hydrogen phthalate solution.

The pH-measurements were performed with a Metrohm 211 microprocessor (Hanna, Romania). The microprocessor and electrode were calibrated with standard buffer solutions, prepared according to NBS specification¹⁴.

The dissociation constants of the sulfanilamide and other ligands were determined potentiometrically by titrating the ligand (40 mL) solution (1×10^{-2} mol/L). The stability constant of the Cu(II)-sulfanilamide complex was determined using potentiometric data obtained from mixtures (40 mL) of copper ion (5×10^{-3} mol/L) and sulfanilamide in concentration ratios of 1:1 and 1:2. The stability constants of the ternary complexes were determined by titrating 40 mL of a solution mixture contained equivalent amounts of copper(II) ion, S and the other ligand (1×10^{-2} mol/L). All titrations were performed in a purified N₂ atmosphere at I = 0.1 mol/L NaNO₃, using aqueous 0.01 mol/L NaOH as titrant.

Data processing: The stoichiometries and stability constants of the complex species formed in aqueous solution were determined through the study of various possible composition models for the systems studied. All the dissociation and the complex formation constants were determined by using the computer program HYPERQUAD¹⁵ and the speciation as a function of pH using the computer program HYSS¹⁶.

RESULTS AND DISCUSSION

The acid dissociation constants of the ligands, the formation constants of the Cu(II)-sulfanilamide and Cu(II)-L (L = amino acid or peptide) complexes and the formation constants of the ternary complexes were determined in a aqueous solution at 25 °C and ionic strength 0.1 mol/L NaNO₃ and the results are given in Table-1.

TABLE-1
STABILITY CONSTANTS OF BINARY SYSTEMS Cu(II)-S,
Cu(II)-L AND PROTON-ASSOCIATION CONSTANTS
AT 25 °C AND I = 0.1 mol/L NaNO₃

System	l	p	q	r ^a	log ₁₀ β ^b
Sulfanilamide (S)	0	1	0	1	10.51 (0.006)
	0	1	0	2	12.84 (0.009)
	1	1	0	0	7.00 (0.02)
	1	1	0	-1	-3.99 (0.07)
Glycine	0	0	1	1	9.64 (0.01)
	0	0	1	2	12.17 (0.02)
	1	0	1	0	8.17 (0.02)
	1	0	2	0	14.93 (0.04)
Alanine	0	0	1	1	9.80 (0.01)
	0	0	1	2	12.62 (0.03)
	1	0	1	0	8.03 (0.03)
	1	0	2	0	14.77 (0.05)
Valine	0	0	1	1	9.68 (0.00)
	0	0	1	2	12.18 (0.01)
	1	0	1	0	8.11 (0.02)
	1	0	2	0	14.73 (0.03)
Proline	0	0	1	1	10.65 (0.009)
	0	0	1	2	13.18 (0.01)
	1	0	1	0	8.60 (0.03)
	1	0	2	0	15.97 (0.05)
β-Phenyl alanine	0	0	1	1	9.20 (0.01)
	0	0	1	2	11.81 (0.03)
	1	0	1	0	7.69 (0.02)
	1	0	2	0	14.25 (0.03)

Methionine	0	0	1	1	9.23 (0.02)
	0	0	1	2	12.04 (0.04)
	1	0	1	0	7.72 (0.03)
	1	0	2	0	14.16 (0.05)
Isoleucine	0	0	1	1	9.82 (0.01)
	0	0	1	2	12.46 (0.04)
	1	0	1	0	8.19 (0.02)
	1	0	2	0	15.03 (0.03)
Glutamic acid	0	0	1	1	9.46 (0.01)
	0	0	1	2	13.54 (0.01)
	1	0	1	0	8.26 (0.01)
	1	0	2	0	15.61 (0.02)
Threonine	0	0	1	1	9.06 (0.009)
	0	0	1	2	11.07 (0.03)
	1	0	1	0	8.34 (0.02)
	1	0	2	0	14.80 (0.04)
Serine	0	0	1	1	9.17 (0.01)
	0	0	1	2	11.54 (0.03)
	1	0	1	0	8.04 (0.02)
	1	0	2	0	14.54 (0.04)
Ornithine	0	0	1	1	10.47 (0.03)
	0	0	1	2	19.27 (0.04)
	0	0	1	3	20.98 (0.05)
	1	0	1	0	11.85 (0.04)
	1	0	2	0	15.95 (0.07)
	1	0	1	1	17.69 (0.03)
Cystine	0	0	1	1	9.68 (0.01)
	0	0	1	2	17.72 (0.02)
	0	0	1	3	19.35 (0.06)
	1	0	1	0	15.12 (0.03)
	1	0	2	0	19.50 (0.06)
Histidine	0	0	1	1	9.48 (0.01)
	0	0	1	2	15.76 (0.01)
	0	0	1	3	17.92 (0.04)
	1	0	1	0	10.65 (0.01)
	1	0	2	0	18.68 (0.03)
Histamine	0	0	1	1	9.88 (0.03)
	0	0	1	2	15.94 (0.05)
	1	0	1	0	9.39 (0.02)
	1	0	2	0	15.12 (0.05)
Penicillamine	0	0	1	1	10.41 (0.02)
	0	0	1	2	18.29 (0.03)
	0	0	1	3	19.55 (0.09)
	1	0	1	0	15.71 (0.04)
	1	0	2	0	29.02 (0.06)
Imidazol	0	0	1	1	7.06 (0.01)
	1	0	1	0	4.23 (0.01)
	1	0	2	0	7.57 (0.02)
Mercaptoethanol	0	0	1	1	9.52 (0.01)
	0	0	1	2	12.16 (0.02)
	1	0	1	0	11.61 (0.01)
Methylamine	0	0	1	1	10.07 (0.02)
	1	0	1	0	6.80 (0.01)
	1	0	2	0	10.54 (0.02)
Glycylglycine	0	0	1	1	8.26 (0.009)
	0	0	1	2	11.44 (0.02)
	1	0	1	0	5.64 (0.01)
Glycinamide	0	0	1	1	8.06 (0.01)
	1	0	1	0	4.37 (0.01)
Glutamine	0	0	1	1	8.99 (0.03)
	1	0	1	0	7.07 (0.04)

^al, p and q are the stoichiometric coefficient corresponding to Cu(II), S (or bioligands) and H⁺, respectively. ^bStandard deviations are given in parentheses.

Dissociation constants of sulfanilamide: Sulfanilamide (S) has two groups: the first protonation constant refers to the amino group of the sulfonamide group ($pK_{a1} = 10.51$) and the second to the amino group of the aniline group ($pK_{a2} = 2.33$). These results obtained are compliant with previous investigations undertaken for related systems¹⁷.

Stability constant of binary complexes: In the binary systems, the titration curve of the Cu(II)-sulfanilamide complex is significantly lower than the sulfanilamide titration curve, indicating formation of Cu(II) complexes by displacement of protons (Fig. 2). Beside, the potentiometric titration data of the binary complex formation equilibria were fitted to various models. The selected model with the best statistical fit was found to consist of Cu(S) and Cu(S)H₁ complexes. While in case of copper with bioligand, the selected model was found to consist of Cu(L), Cu(L)₂ and Cu(L)₃ species. Fig. 3 represents the speciation diagram of the binary Cu(II) complex with S. The complex species with coefficients 110 [Cu(S)] reaches the maximum degree of formation (99.15 %) at pH = 8.474. However, the species 11-1 [Cu(S)H₁] attains a maximum concentration of 90.37 % at pH = 11.96.

Fig. 2. Potentiometric titration curve of the Cu(II)-S system

Fig. 3. The concentration distribution diagram of the Cu-S system

Ternary complexes involving Cu²⁺, sulfanilamide and bioligands: In general, the overall formation constant can be written as follows (charges are omitted for simplicity)

$$\beta_{ipqr} = \frac{[\text{Cu}_1\text{S}_p\text{L}_q\text{H}_r]}{[\text{Cu}]^1[\text{S}]^p[\text{L}]^q[\text{H}]^r}$$

Ternary complexes of amino acids: The formation constants of 1:1 Cu(II) complexes with S or L are of the same order of magnitude (Table-1). Consequently the ligation of sulfanilamide and L will proceed simultaneously. The titration data of the ternary complexes with sulfanilamide and amino acids fit satisfactorily with formation of the species: Cu(S), Cu(S)H₁, Cu(L), Cu(L)₂, Cu(L)H, Cu(S)(L), Cu(S)(L)H and Cu(S)(L)H₁. The stability constants of the amino acids complexes are larger than those for the corresponding monodentate methylamine and imidazole complexes, indicating that amino acids most likely coordinates with Cu(II)-S as a bidentate ligand *via* the amino and carboxylate groups, rather than as a monodentate ligand. Ornithine has two amino and one carboxylic group. From Table-1, the stability constants of mixed ligand complexes of ornithine are larger than those of α -amino acids. It can be understood that ornithine most likely chelates *via* the two amino groups. Unlike ornithine, the stability constant of the glutamic acid complex is in fair agreement with those of amino acids. This clearly implies that glutamic acid most likely chelates through the amino and carboxylate groups. Threonine and serine forms the Cu(S)(L), Cu(S)(L)H and Cu(S)(L)H₁ species. The latter complex is formed through induced ionization of the β -alcohol group as mentioned in the literature¹⁸.

Histidine has three binding sites amino, imidazole and carboxylate groups. Our results show that the stability constant of the histidine complex is larger than that of α -amino acids and close to that of histamine (Table-1). This reveals that histidine interacts with copper *via* the amino and imidazole nitrogen atoms (histamine-like). The acid dissociation constant of the protonated species is given by the following eqn. 1

$$pK_{\text{Cu(S)L}}^{\text{H}} = \log_{10} K_{\text{Cu(S)(L)H}}^{\text{Cu(S)}} - \log_{10} K_{\text{Cu(S)(L)}}^{\text{Cu(S)}}$$

In this regard, the pK_a for the histidine complex amounts to 6.92, lower than that of the protonated amino group NH_3^+ ($pK_a = 9.48$), but closer to that of the protonated imidazole group ($pK_a = 6.28$), thereby suggesting the proton in the protonated complex would be located mainly on the imidazole group.

Penicillamine and cystine are potentially a tridentate ligand with carboxylic, amino and sulfhydryl groups as copper ion binding sites. They form the complexes 1110, 1111 and 111-1. The stability constant of the 1110 complex are larger of mercaptoethanol (where the binding sites are the carboxylic and sulfhydryl groups) and much larger than those for α -amino acids (where the binding sites are the amino and sulfhydryl groups). This clearly implies that the penicillamine and cystine interact with Cu(II) ion *via* the amino and deprotonated-SH groups.

Ternary complex of peptides: The titration data of the ternary complexes with peptides and sulfanilamide fit satisfactorily with formation of the species: Cu(L), Cu(S), Cu(S)H₁, Cu(S)(L), Cu(S)(L)H and Cu(S)(L)H₁. Our results show that the stability of the complex with glycylglycine is higher than glycylglycine as indicated due to the negative charge of glycylglycinate compared to neutral glycylglycine. Consequently, the electrostatic interaction between the di-positively charged copper(II) complex and the negatively charged glycylglycine will result in a decreasing of the Gibbs energy of formation. Fig. 4, shows the distribution diagram for the glycylglycine

Fig. 4. Concentration distribution of various species with pH in the ternary Cu(II)-S-glycylglycine system

complex. The deprotonated 1110 complex species predominates with a formation degree amounting to 97.84 % at pH = 7.389, whereas the 111-1 species attains a maximum concentration of 99.19 % at pH = 11.79. Therefore, the species 1110 predominates in the physiological pH range.

The amide groups undergo deprotonation and the Cu(S)(L)H₁ complexes are formed. The pK^H values are calculated by the following equation

$$pK_{Cu(S)L}^H = \log_{10} K_{Cu(S)(L)}^{Cu(S)} - \log_{10} K_{Cu(S)(L)H_2}^{Cu(S)}$$

The pK_a of the amide group for glycylglycine, glycylamide and glutamine are 9.87, 7.73 and 10.08 respectively. It is clear that, the pK_a value for glycylamide complex is lower than those for other peptides. This may be due to the bulky substituent group on the other peptides that may hinder the structural changes when going from species 1110 to 111-1 (peptide ionization).

Δ log₁₀ K: The parameter Δ log K values are generally used to indicate the relative stability of the ternary complexes as compared to the binary ones as in the following equations:

$$\Delta \log_{10} K = \log_{10} \beta_{Cu(S)L}^{Cu(S)} - (\log_{10} \beta_{Cu(S)}^{Cu} + \log_{10} \beta_{Cu(L)}^{Cu})$$

The theoretical Δ log₁₀ K value for a square-planer copper(II) complex is -0.9¹⁹. The negative values of the bio ligands are less than the theoretical value (-0.9), thereby this may be considered as evidence for the occurrence of enhanced stabilities involving π-back-donation from the negatively charged amino acid to the π-system of the sulfanilamide. In contrast, the positive values of Δ log₁₀ K in this paper indicate that the ternary complexes are more stable than the binary complexes²⁰ (Table-2). Probably due to intramolecular aromatic-ring staking, hydrogen bond and π-π cooperative effect between ligands.

Conclusion

Sulfanilamide (S), an antimicrobial, forms a reasonably stable complex with Cu²⁺ and some bioligand in aqueous solution at 25 °C and ionic strength 0.1 mol/L NaNO₃. Also, the relative stabilities of each ternary complexes are compared with those of the corresponding binary complexes. Finally, the concentration distribution curves of the various complex species existing in solution were evaluated.

TABLE-2
STABILITY CONSTANTS OF THE TERNARY SPECIES IN THE Cu(II)-S-L SYSTEMS AT 25 °C AND I = 0.1 mol/L NaNO₃

System	l	p	q	r ^a	log ₁₀ β ^b	Δlog K
Glycine	1	1	1	0	15.13 (0.05)	-0.04
	1	1	1	1	22.12 (0.04)	
	1	1	1	-1	4.73 (0.06)	
Glycine	1	1	1	0	15.13 (0.05)	-0.04
	1	1	1	1	22.12 (0.04)	
	1	1	1	-1	4.73 (0.06)	
Alanine	1	1	1	0	14.95 (0.04)	-0.08
	1	1	1	1	22.04 (0.03)	
	1	1	1	-1	4.46 (0.05)	
Valine	1	1	1	0	15.56 (0.03)	0.45
	1	1	1	1	22.66 (0.02)	
	1	1	1	-1	5.08 (0.03)	
Proline	1	1	1	0	15.25 (0.02)	-0.35
	1	1	1	1	22.20 (0.02)	
	1	1	1	-1	4.84 (0.03)	
β-Phenyl alanine	1	1	1	0	16.74 (0.03)	2.05
	1	1	1	1	23.10 (0.03)	
	1	1	1	-1	6.43 (0.03)	
Methionine	1	1	1	0	14.72 (0.02)	0.00
	1	1	1	1	21.98 (0.02)	
	1	1	1	-1	3.56 (0.03)	
Isoleucine	1	1	1	0	15.71 (0.09)	0.52
	1	1	1	1	23.43 (0.02)	
	1	1	1	-1	9.84 (0.02)	
Glutamic acid	1	1	1	0	15.14 (0.02)	-0.13
	1	1	1	1	21.45 (0.03)	
	1	1	1	-1	5.38 (0.03)	
Threonine	1	1	1	0	15.58 (0.02)	0.24
	1	1	1	1	22.12 (0.02)	
	1	1	1	-1	4.47 (0.02)	
Serine	1	1	1	0	15.15 (0.03)	0.11
	1	1	1	1	22.24 (0.03)	
	1	1	1	-1	4.77 (0.04)	
Ornithine	1	1	1	0	18.86 (0.04)	0.01
	1	1	1	1	26.75 (0.03)	
	1	1	1	-1	9.16 (0.07)	
Cystine	1	1	1	0	23.90 (0.03)	1.78
	1	1	1	1	28.29 (0.05)	
	1	1	1	-1	13.14 (0.03)	
Histidine	1	1	1	0	17.57 (0.02)	-0.08
	1	1	1	1	24.49 (0.02)	
	1	1	1	-1	6.76 (0.02)	
Histamine	1	1	1	0	17.79 (0.02)	1.4
	1	1	1	1	23.60 (0.01)	
	1	1	1	-1	9.45 (0.03)	
Penicillamine	1	1	1	0	24.29 (0.04)	1.58
	1	1	1	1	28.93 (0.06)	
	1	1	1	-1	16.79 (0.04)	
Imidazol	1	1	1	0	11.19 (0.01)	-3.38
	1	1	1	-1	3.31 (0.01)	
Mercaptoethanol	1	1	1	0	19.01 (0.06)	0.4
	1	1	1	1	25.70 (0.08)	
	1	1	1	-1	7.15 (0.10)	
Methylamine	1	1	1	0	13.80 (0.01)	0.00
	1	1	1	-1	2.77 (0.03)	
Glycylglycine	1	1	1	0	14.53 (0.01)	1.89
	1	1	1	-1	4.66 (0.01)	
Glycinamide	1	1	1	0	11.64 (0.01)	0.27
	1	1	1	-1	3.91 (0.01)	
Glutamine	1	1	1	0	13.85 (0.04)	-0.22
	1	1	1	1	20.85 (0.04)	
	1	1	1	-1	3.77 (0.06)	

^al, p and q are the stoichiometric coefficient corresponding to Cu(II), S, (bioligands) and H⁺, respectively; ^bStandard deviations are given in parentheses.

ACKNOWLEDGEMENTS

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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