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Cu(II), Co(II), and Ni(II)−Antioxidative Phenolate−Glycine Peptide Systems: An Insight into Its Equilibrium Solution Study

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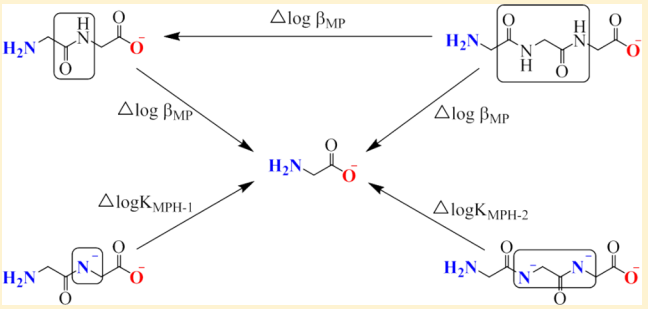
ABSTRACT: The stability of complex formation between divalent transition metal ions, phenolates and glycine peptides was studied at 298.15 K in aqueous solution with an ionic strength of 0.15 mol·dm^{-3} $NaNO_3$. HYPERQUAD 2008, a program based on nonlinear least-squares curve fitting, was used to determine the stability constants of the complexes formed from the pH-potentiometric data. The trends in stability constants of the complexes and the contribution of deprotonated or undeptotonated amide peptide in the stability constant were discussed. From the stability constants that obtained, the representative species distribution diagrams of copper complexes were provided by the HYSS 2009 program. In addition, structures of the formed complexes were predicted by using the Gaussian 09 program. The Gibbs free energies of these complexes were also evaluated in the simulation.



INTRODUCTION

Phenolates are secondary plant metabolites widely spread throughout the plant kingdom and thought to be an integral part of both human and animal diets.[1] They are commonly present under two principal forms in all plant-derived foods: a free and a bound form. The bound form is found more frequently and occurs in the form of esters, glycosides, and bound complexes.[2] Since they exhibit antioxidative properties, there has been growing interest in the study of phenolates and their derivatives. Many in vivo and in vitro studies indicate that their activities depend on the number and position of hydroxyl groups bound to the aromatic ring, the binding site and mutual position of hydroxyl groups in the aromatic ring, and the type of substituent.[3−6]

It is known that the metal-based glycine peptide scaffold could provide an excellent delivery system into the specific tumor target sites and could be less toxic to normal tissues. Peptide-based drug design is a challenging option to address issues of toxicity and efficacy and chemical strategies that involve therapeutic peptides as scaffolds for drug design, and these are currently gaining much attention for use in cancer chemotherapy. The rationale is that using glycine peptides in complex form will deliver more pronounced biological and better pharmacological activity.[7] Metal ions help to tune the reactivity, and the conformational changes, are catalytically active for many biochemical reactions[8] and provide the mimicry of the active site of metalloenzymes.[9] Also, glycine peptides may act as excellent coordination chelator since it contains many potential donor atoms.[10,11]

The study of complex formation between metal ions and antioxidant ferulic acid (FA) or gallic acid (GA) in water in the presence of some nonproteinogenic amino acids or soluble vitamins has been gained much attention from our research group in the last three years.[12−15] A thorough literature review showed that no work seems to have been done on the study of complexation between metal ions, phenolic acids, and glycine peptides in aqueous solutions. In the present work, the previously studied ligands were used to mimic a biological system in which phenolic acid acts as an antioxidant, while glycine (G) and its small peptides act as building blocks of enzymes or protein that show their importance in biological reactions.[16−20] The amide nitrogen in the peptide backbone is known to be a potential binding site for metal ions; these metal ions will induce the deprotonation of the amide nitrogen in peptide bonds, thus forming a stable chelate ring with the metal ion.[21−24] So, in addition to determining the complex stability constant, it is necessary to study the influence of amide nitrogen deprotonation in glycylglycine (GG) and glycylglycylglycine (GGG) peptide bonds on the complex stability. Herein, the stability constants of divalent metal ion−phenolic acid (A = FA, GA)−glycine peptide (P = G, GG, GGG) complexes in binary and mixed ligand systems were determined by pH-potentiometric titrations in aqueous solutions. The measurements were completed at 298.15 K in solutions with an ionic strength of 0.15 mol·dm^{-3} $NaNO_3$, and the data analyses were done using the HYPERQUAD 2008 computational program.

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EXPERIMENTAL SECTION

Materials and Solutions. All chemicals were analytical grade and were used without further purification. Ferulic acid ($C_{10}H_{10}O_4$, 0.99 purity) was purchased from Aldrich (Germany). Gallic acid ($C_7H_6O_5$, 0.98 purity) was obtained from Tokyo Chemical Industry (Japan). Glycine-containing peptides (G, GG, and GGG) used were analytical grade chemicals with a purity of 0.99 from Sigma (Germany). Nitric acid (Panreac, Spain) solution was prepared and standardized before used. Copper chloride dihydrate ($CuCl_2{\cdot}2H_2O$, 0.99 purity) was a product of Kanto Chemical Co., Inc. (Japan). Nickel chloride hexahydrate ($NiCl_2{\cdot}6H_2O$, 0.97 purity) and cobalt nitrate hexahydrate ($Co(NO_3)_2{\cdot}6H_2O$, 0.99 purity) were obtained from Acros Organics (USA). Sodium nitrate ($NaNO_3$, 0.99 purity) from Acros Organics (USA) was used. Carbonate-free sodium hydroxide solution was prepared by dissolving a NaOH pellet (Across Organics, USA) with ultrapure water, and the solution was potentiometrically standardized against potassium hydrogen phthalate with a purity of 0.9995 (Aldrich, USA). All solutions were freshly prepared daily. Chemicals were accurately weighed and then dissolved in ultrapure water (NANOpure-Ultrapure water that deionized with 18.3 $M\Omega{\cdot}cm^{-1}$ resistance and distilled).

pH-Potentiometric Titration. The pH-potentiometric measurements of glycine peptides (P) in the absence or presence of divalent metal ions and FA and GA ligands were performed using a Metrohm 702 SM titrator, equipped with 664 Dosimate, a 728 magnetic stirrer, and coupled with Dosino buret model 683. The electrode response can be read to the third decimal place in terms of pH units with a precision of ± 0.001. Before each titration, the pH-meter was calibrated with Metrohm buffer standard solution at pH 4.00, 7.00, and 9.00. The buret's volume droplet calibration was also carried out daily to increase the experiment accuracy.

All pH measurements were carried out in a 100 cm^3 double-walled equilibrium cell in which the temperature and ionic strength of the solutions were maintained at 298.15 K (by a refrigerated circulating bath) and 0.15 $mol{\cdot}dm^{-3}$ (by using $NaNO_3$ solution), respectively. Titrations for metal−ligand complex stability determination were done at various concentration ratios as shown in Table 1. Each titration was repeated at least three times with a reproducibility of ± 0.02 in the pH range ~2.5 to 11.

Data Analysis. The HYPERQUAD 2008 program was used to obtain protonation and complex stability constants from potentiometric data.[25] For this purpose, a fitting criterion based on the minimization of the nonlinear least-squares sum defined by the difference between the calculated and the experimental data of the titration curves was used.

$$x^2 = \frac{\sum (E_{cal} - E_{exp})^2}{E_{cal}}$$

These constants were presented as log value of the overall formation constant (log β_{pqrs}) and expressed by the following equation:

$$pM + qA + rP + sH \rightleftharpoons M_pA_qP_rH_s$$

$$\beta_{pqrs} = \frac{[M_pA_qP_rH_s]}{[M]^p[A]^q[P]^r[H]^s}$$

The HYPERQUAD 2008 program permits the formation constant determination of different complex species that can be formed in the aqueous solution simultaneously.[26] Various models with a possible composition of formed complex were proposed in the program, and the model that gave the best statistical fit and chemically sensible was chosen. Besides HYPERQUAD 2008, the Hyperquad Simulation and Speciation (HySS 2009) program is also used for providing speciation diagrams to present the distributions of various complex species that formed in electrolyte solutions and furnishes a variety of data presentations, including tables of concentrations of all species present in solution in the selected pH ranges.[27]

In this work, the Gaussian 09 program was used to predict the structure of the complexes.[28] The model structure of these complexes were optimized using density functional theory (DFT) with the Becke's three-parameter hybrid[29] with the correlation of Lee−Yang−Parr, LYP (B3LYP), method,[30] and the 6-31+G(d) was used as basis set.[31−33] Along with the geometry optimization, the frequency analysis was done to obtain the thermochemical properties of the complexes. The B3LYP/6-31+G(d) basis set was chosen since it has been shown as one of the suitable basis sets to approach the molecular orbital of metal complexes.[31−35] The validity of the structure optimization was checked by using normal-mode frequency analysis, in which the real minimum structure must exhibit a positive value for all frequencies. The modeling was performed by employing a number of assumptions as simplification, such as the usage of fully deprotonated ligands, only considering the complex formation of one metal ion and one ligand, not considering the usage of salt to maintain the solution's ionic strength and not considering the addition of acid/base during the potentiometric titration.

Table 1. Metal and Ligand Concentrations Used To Determine the Overall Formation Constants of the Complexes at 298.15 K and 0.15 $mol{\cdot}dm^{-3}$ $NaNO_3$

	ratios	T_M
systems	T_M:T_L	$mol{\cdot}dm^{-3}$
L[a]		$1{\cdot}10^{-3}$
M:L	1:1	$1{\cdot}10^{-3}$
	1:2	$5{\cdot}10^{-4}$
	1:3	$4{\cdot}10^{-4}$
$M:L_P:L_A$[b]	1:1:1	$1{\cdot}10^{-3}$

[a]Only in this system, T_M represents the ligand total concentration in the solution. [b]P: peptide; A: phenolic acids.

RESULTS AND DISCUSSION

Complex Formation between Divalent Metal Ions and Glycine Peptide. From the refinement of pH-potentiometric result by the HYPERQUAD 2008 program, the protonation constants as overall formation constants (log β) were determined.

$$H^+ + P^- \rightleftharpoons HP^{\pm} \qquad [HP] = \beta_1[H^+][P^-]$$

$$2H^+ + P^- \rightleftharpoons H_2P^+ \qquad [H_2P^+] = \beta_2[H^+]^2[P^-]$$

These constants are then presented as the minus logarithm of a stepwise acid dissociation constant (pK_a) by the following relation:

$$H_2P^+ \rightleftharpoons H^+ + HP^{\pm} \qquad [H^+][HP] = K_{a1}[H_2P^+]$$

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$$HP^{\pm} \rightleftharpoons H^+ + P^- \qquad [H^+][P^-] = K_{a2}[HP]$$

where log β_1 refers to the second pK_a of the ligand and log β_2 equals the summation of the first and the second pK_a. The charge of the first deprotonated form (HP) of the ligand is symbolized as ± to show their zwitterionic property.

The pH-potentiometric curve of glycine peptides in the absence of metal ions is depicted in Figure 1, and their

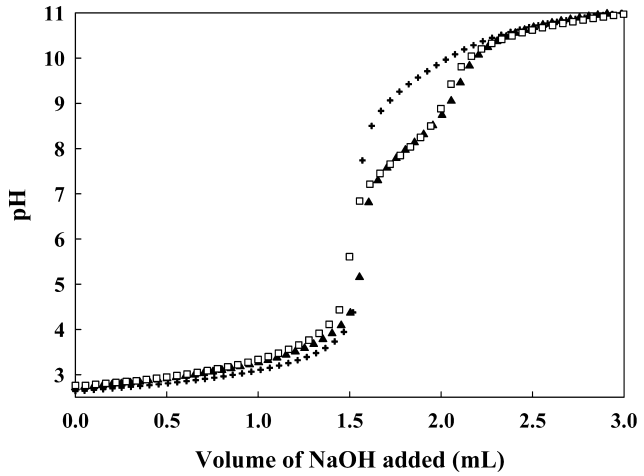

Figure 1. Potentiometric titration curve for the protonation constant determination of glycine peptides (+, glycine; ▲, glycylglycine; □, glycylglycylglycine) at T = 298.15 K and I = 0.15 mol·dm^{-3} $NaNO_3$.

Table 2. Protonation Constants of Glycine Peptides in Water at 298.15 K and 0.15 mol·dm^{-3} $NaNO_3$

ligand	pK_{a1}	pK_{a2}
glycine	2.32 ± 0.01 (2.33)[a]	9.62 ± 0.01 (9.61)[a]
glycylglycine	3.21 ± 0.01 (3.16)[b]	8.13 ± 0.01 (8.15)[b]
glycylglycylglycine	3.30 ± 0.01 (3.26)[c]	7.90 ± 0.01 (7.93)[c]

[a]Reference 36, T = 298.15 K, I = 0.05 mol·dm^{-3} KCl. [b]Reference 37, T = 298.15 K, I = 0.1 mol·dm^{-3} KNO_3. [c]Reference 38, T = 298.15 K, I = 0.1 mol·dm^{-3} KNO_3.

protonation constants as pK_a values are reported in Table 2 along with the previous values of some reported literature.[36−38] By comparing our calculated values with the literature values, we found an adequate difference and explained it in regard to the variance of the experimental condition. The stepwise acid dissociation values of glycine and its peptides are depicted in Scheme 1, in which the first deprotonation occurred in the carboxyl group of C-terminus and followed by the deprotonation of amine group in the N-terminus.

These protonation constants were introduced into the HYPERQUAD 2008 program as a constant and used for the determination of complex stability between divalent metal ions (Cu^{2+}, Ni^{2+}, Co^{2+}) and glycine peptides (P). The stability of the metal complex is expressed as the overall formation constant. The overall formation constant values of various metal ions (Cu^{2+}, Ni^{2+}, Co^{2+}) and peptides (P) are presented in Table 3. Among the metal ions studied, Cu^{2+} formed the most stable complex with glycine peptides, followed by Ni^{2+} and Co^{2+}. This trend was observed regardless of the peptide used (P = G, GG, or GGG). This trend is in agreement with the Irving−Williams series[39] that is based on the ionic radius of the metal ions, crystal field stabilization energy, and Jahn−Teller distortion effect.

For a fixed metal ion, glycine formed the most stable complex with this metal ion, followed by a glycylglycine metal complex and then by glycylglycylglycine metal complex. From previously reported literature,[10,21,22,40] the amide nitrogen in peptide bond shows a deprotonation activity thus affecting the stability of the complex species formed in the solutions. In this study, the influence of the peptide bond in its undeprotonated and deprotonated forms were compared and investigated by the following expressions:

$$M + P \rightleftharpoons MP \qquad [MP] = \beta_{MP}[M][P]$$

$$M + P \rightleftharpoons MPH_{-1} + H$$

$$[MPH_{-1}] = \beta_{MPH-1}[M][P]/[H]$$

$$M + P \rightleftharpoons MPH_{-2} + 2H$$

$$[MPH_{-2}] = \beta_{MPH-2}[M][P]/[H]^2$$

$$MP \rightleftharpoons MPH_{-1} + H$$

$$[MPH_{-1}] = K_{MPH_{-1}}[MP]/[H]$$

$$\beta_{MPH_{-1}} = K_{MPH_{-1}}\beta_{MP}$$

$$MP \rightleftharpoons MPH_{-2} + 2H$$

$$[MPH_{-2}] = \beta_{MPH_{-2}}[MP]/[H]$$

$$\beta_{MPH_{-2}} = K_{MPH_{-2}}\beta_{MP}$$

where in glycine metal complex, a negative stoichiometric value for H refers to the hydroxo-complex, while in GG and GGG metal complexes, it refers to the deprotonation of the amide nitrogen in the peptide bond. For example, the MPH_{-1} in the

Scheme 1. Protonation Equilibria of (a) Glycine, (b) Glycine Small Peptide, n = 1 for Glycylglycine (GG); n = 2 for Glycylglycylglycine (GGG)

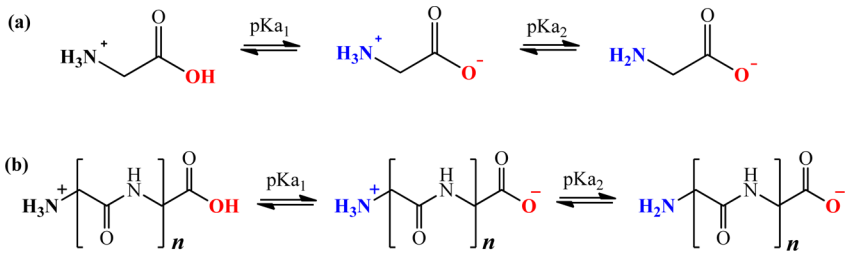

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Table 3. Overall Formation Constants of Divalent Metal Ion (M)−Glycine Peptide (P) Complexes at 298.15 K and 0.15 mol·dm^{-3} $NaNO_3$

species	log β_{pqrs} ± SD					
	Cu(II)		Ni(II)		Co(II)	
			P = Glycine			
$[MP]^+$	8.28 ± 0.01	(8.22)[a]	5.74 ± 0.02	(5.80)[a]	5.03 ± 0.02	(5.07)[d]
$[MP_2]$	15.38 ± 0.01	(15.11)	10.41 ± 0.11	(10.65)	8.97 ± 0.06	(9.04)
$[MP_3]^-$	19.55 ± 0.03		14.95 ± 0.02			
$[MPH_{-1}]$	1.28 ± 0.01		−3.19 ± 0.02		−4.19 ± 0.05	
$[MPH_{-2}]^-$	−9.25 ± 0.02		−13.51 ± 0.03		−13.74 ± 0.02	
$[MP_2H_{-1}]^-$	4.91 ± 0.05					
$[MP_2H_{-2}]^{2-}$	−4.96 ± 0.04					
			P = Glycylglycine			
$[MP]^+$	5.60 ± 0.05	(5.68)[b]	4.33 ± 0.04	(4.11)[c]	3.62 ± 0.01	
$[MPH_{-1}]$	1.58 ± 0.01	(1.50)	7.55 ± 0.08	(7.32)	−5.19 ± 0.01	
$[MPH_{-2}]^-$	−7.38 ± 0.01		−3.66 ± 0.03			
$[MP_2H_{-1}]^-$	5.29 ± 0.02					
$[MP_2H_{-2}]^{2-}$	−4.02 ± 0.03					
			P = Glycylglycylglycine			
$[MP]^+$	5.34 ± 0.02	(5.24)[c]	4.05 ± 0.02		3.62 ± 0.02	
$[MPH_{-1}]$	0.01 ± 0.01	(0.02)	7.16 ± 0.09		−5.11 ± 0.07	
$[MPH_{-2}]^-$	−6.52 ± 0.01	(−6.58)	−4.27 ± 0.06		−13.64 ± 0.05	
$[MP_2H_{-1}]^-$	4.59 ± 0.03		−12.43 ± 0.03			
$[MP_2H_{-2}]^{2-}$	−2.65 ± 0.02					

[a]Reference 36, T = 298.15 K, I = 0.05 mol·dm^{-3} KCl. [b]Reference 37, T = 298.15 K, I = 0.1 mol·dm^{-3} KNO_3. [c]Reference 47, T = 298.15 K, I = 0.1 mol·dm^{-3} KNO_3. [d]Reference 48, T = 298.15 K, I = 0.

system containing glycylglycine refers to the formation of the complex between a metal ion with this ligand in its amide deprotonated form (GGH_{-1}). The subtraction of log β_{MP} of glycine from those of diglycine and triglycine as well as the subtraction of log β_{MP} of diglycine from that of triglycine were carried out to evaluate the effect of the undeprotonated amide nitrogen peptide bond. Similarly, the impact of amide nitrogen deprotonation to the stability of the metal complexes was evaluated by carrying out the subtraction of log $K_{MPH_{-1}}$ and log $K_{MPH_{-2}}$ of G from that of GG and GGG, respectively, as illustrated in Scheme 2.

The values of log β_{MP} subtraction are listed in Table 4. The negative values indicate the absence of undeprotonated amide nitrogen contribution in the complexation of metal ions with the G, GG, and GGG ligands. It is interesting to note that the $\Delta\log\beta_{MP}$ values of metal complexes involving GG and GGG are

Scheme 2. Influence of (a) Peptide Bond and (b) Deprotonated Amide Group in the Complex Stability

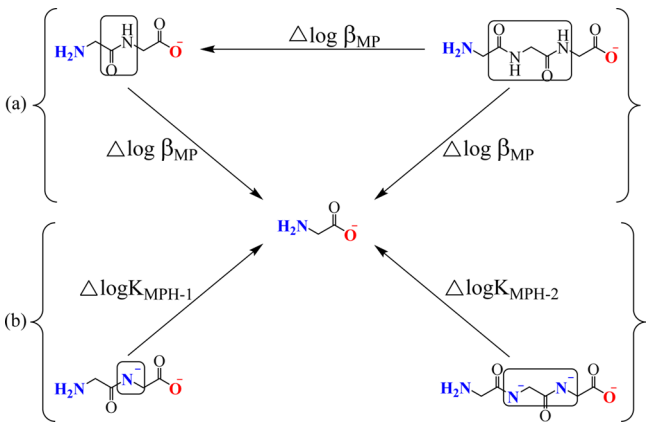

Table 4. Influence of Undeprotonated Amide Nitrogen in Peptide Bond on Stability of Metal - Peptide Complex

metal ion	Δ log β_{MP}		
	GG−G	GGG−GG	GGG−G
Cu(II)	−2.67	−0.27	−2.94
Ni(II)	−1.42	−0.28	−1.70
Co(II)	−1.41	0	−1.41

very small (0.26 for Cu^{2+} system and 0.28 for Ni^{2+} system) and zero for the Co(II) system (Table 4) while subtraction values of the logβ_{MP} of GG-G and GGG-G vary from −1.41 to −2.94. The phenomena can be explained from the structure of the peptide ligands itself. Glycine, the smallest and the simplest ligand can form five-membered ring complex with the metal ion via deprotonated amine and carboxyl group of the amino acid while for the GG and GGG, the metal ions will form complex with the deprotonated amine group in the N-terminus and form less stable chelate to the peptide oxygen.[10] The small $\Delta\log\beta_{MP}$ values of GGG-GG occurred due to the extensive backbone length of GGG as a ligand thus led to the occurrence of steric effect.[41,42] But since these two ligands (GG and GGG) form chelates in the same manner, the stability of the complex formed are also similar.

On the other hand, as shown in Table 5, Δ log $K_{MPH_{-1}}$ and Δ log $K_{MPH_{-2}}$ are positive which indicate that deprotonated amide nitrogens participate in the complex formation of metal ions. Δ log $K_{MPH_{-2}}$ is more positive than Δ log $K_{MPH_{-1}}$ due to the contribution of two deprotonated amide in GGG peptide in which the metal ion and GGG complex forms three five-membered chelate rings, while in the metal ion−GG system the complex forms two five-membered chelate rings via nitrogen in the N-terminus, deprotonated amide nitrogens of the peptide, and carboxyl group in the C-terminus.

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Table 5. Influence of Deprotonated Amide Nitrogen in Peptide Bond on the Stability of the Metal−Peptide Complex

metal ion	$\Delta \log K_{MPH_{-1}}$ GG−G	$\Delta \log K_{MPH_{-2}}$ GGG−G
Cu(II)	2.98	3.99
Ni(II)	0.95	2.16
Co(II)	0.42	1.01

The phenomena of amide nitrogen contribution in the complex stability were further supported by the optimization and frequency analysis using Gaussian 09. From the geometry optimization, the structure of the complexes that formed can be predicted (Figure 2). The thermochemical properties such as Gibbs free energy (G) can be obtained from the frequency analysis. In this program, the complex equilibrium is presented as the Gibbs free energy of reaction ($\Delta_r G$) that is calculated from the following relations:

$$M^{2+} + L^{-} \rightleftharpoons [ML]^{+}$$

$$\Delta_r G = \sum G_{product} - \sum G_{reactant}$$

$$\Delta_r G = -RT \ln K_c$$

According to the correlation shown above, the more negative the $\Delta_r G$ value is, the larger the equilibrium constant (K_c) value will be, and the complex that is formed will be more stable. From the calculated value of $\Delta_r G$ shown in Table 6, the $\Delta_r G$ negativity and complex stability decreased in the following order: $[MGGGH_{-2}]^{-} > [MGGH_{-1}] > [MGGGH_{-1}] > [MG]^{+} > [MGG]^{+} \geq [MGGG]^{+}$. The optimized geometry of $[MGGH_{-1}]$ and $[MGGGH_{-2}]^{-}$ (shown in Figure 2c and f, respectively) and the calculated values of $\Delta_r G$ supporting the hypothesis that the deprotonated amide in GGG peptide form three five-membered chelate rings with the metal ions, while in metal ion−GG system the complex forms two five-membered chelate rings via nitrogen in the N-terminus, deprotonated amide nitrogens of the peptide, and carboxyl group in the C-terminus.

Formation of Mixed Ligand Complexes. Overall formation constants of ferulic acid and gallic acid in the absence and presence of the metal ions were acquired from the previous research.[12,14] These values together with the overall formation constants of metal ions with G, GG, and GGG were introduced to the HYPERQUAD 2008 program as constants and used for determination of mixed-ligand complex stability involving metal ions, phenolic acids (A), and glycine peptides (P), as reported in Tables 7 and 8. With regard to the metal ions, an identical trend as the Irving−William series[39] was observed in all systems (GA-G, GA-GG, GA-GGG, FA-G, FA-GG, and FA-GGG) in which these ligands bound to the metal ions and formed the most and the least stable complex with copper and cobalt, respectively.

To investigate the stability of the ternary complexes relative to their corresponding binary complexes, $\Delta \log K$ values were calculated using the following equation.[43]

$$\Delta \log K = \log \beta_{MAP} - \log \beta_{MA} - \log \beta_{MP}$$

The calculated values of $\Delta \log K$ are given in Table 9. For all systems that containing gallic acid (GA−G, GA−GG, and GA−GGG) and the FA−G system, the trend of $\Delta \log K$ values for all metal ions is opposite to the stability constant value trend described before in the binary sytem. For example, Cu(II) shows a higher stability constant value than those of Ni(II) and

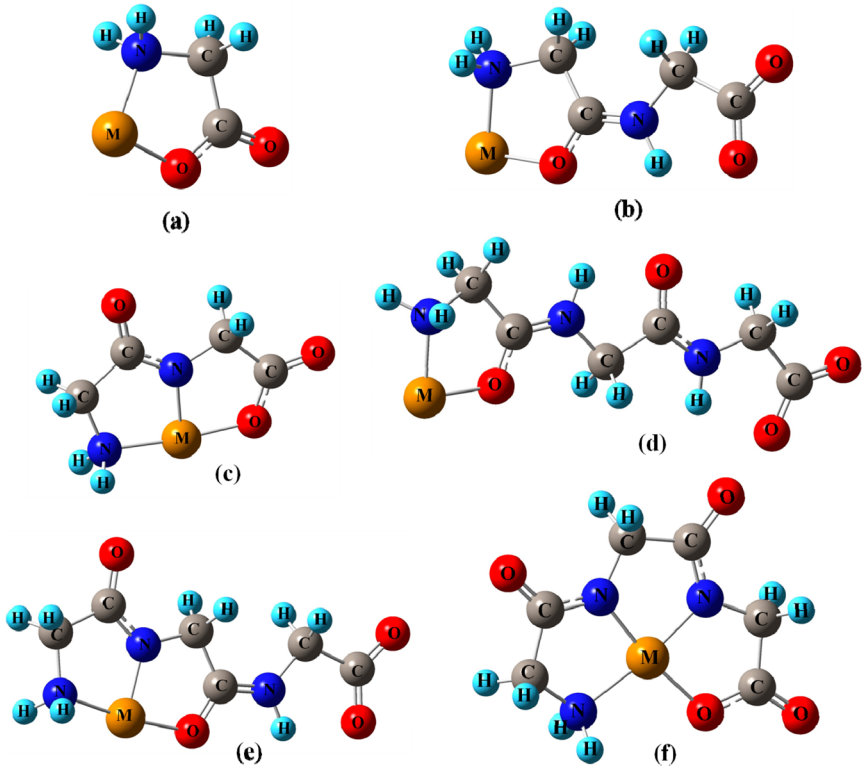

Figure 2. Optimized structures of some complexes between the divalent metal ion (M) and the glycine containing peptides: (a) $[MG]^{+}$, (b) $[MGG]^{+}$, (c) $[M(GGH_{-1})]$, (d) $[MGGG]^{+}$, (e) $[M(GGGH_{-1})]$, and (f) $[M(GGGH_{-2})]^{-}$; generated from the Gaussian 09 calculations.

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Table 6. Gibbs Free Energy of Reaction Obtained from Optimization and Frequency Calculations by Using the Gaussian 09 Program

molecule	G^a hartree·particle^{-1}	$\Delta_r G$ hartree·particle^{-1}
	Metal Ions	
Cu^{2+}	−1639.813789	
Ni^{2+}	−1507.725005	
Co^{2+}	−1382.251038	
	Ligands	
G	−283.956410	
GG	−491.941243	
GGH_{-1}	−491.447298	
GGG	−699.922905	
$GGGH_{-1}$	−699.439247	
$GGGH_{-2}$	−698.936440	
	Cu(II) Complexes	
CuG	−1923.995847	−0.225648
CuGG	−2131.948785	−0.193753
$CuGGH_{-1}$	−2131.584791	−0.323704
CuGGG	−2339.923634	−0.186940
$CuGGGH_{-1}$	−2339.550563	−0.297527
$CuGGGH_{-2}$	−2339.138824	−0.388595
	Ni(II) Complexes	
NiG	−1791.859317	−0.177902
NiGG	−1999.812184	−0.145936
$NiGGH_{-1}$	−1999.449123	−0.276820
NiGGG	−2207.789795	−0.141885
$NiGGGH_{-1}$	−2207.415141	−0.250889
$NiGGGH_{-2}$	−2207.013667	−0.352222
	Co(II) Complexes	
CoG	−1666.343510	−0.136062
CoGG	−1874.301105	−0.108824
$CoGGH_{-1}$	−1873.926051	−0.227715
CoGGG	−2082.272667	−0.098724
$CoGGGH_{-1}$	−2081.911684	−0.221399
$CoGGGH_{-2}$	−2081.484671	−0.297193

[a]The calculation was done using the density functional theory (DFT)-B3LYP method combined with 6-31+G(d) as a basis set, 1 hartree·particle^{-1} = 2.6255048·10^3 kJ·mol^{-1}.

Table 7. Overall Formation Constants of Divalent Metal Ions (M)−Gallic Acid (A)−Glycine Peptide (P) Complexes at 298.15 K and 0.15 mol·dm^{-3} $NaNO_3$

species	log β_{pqrs} ± SD Cu(II)	Ni(II)	Co(II)
	P = Glycine		
$[MAPH_2]^-$	38.34 ± 0.03	33.94 ± 0.02	33.21 ± 0.03
$[MAPH]^{2-}$	32.40 ± 0.03	25.60 ± 0.01	24.79 ± 0.02
$[MAP]^{3-}$	22.25 ± 0.04	15.26 ± 0.02	14.69 ± 0.03
	P = Glycylglycine		
$[MAPH_2]^-$	37.38 ± 0.03		
$[MAPH]^{2-}$	31.13 ± 0.04	24.52 ± 0.01	23.90 ± 0.01
$[MAP]^{3-}$	22.69 ± 0.04	14.79 ± 0.02	14.22 ± 0.02
$[MAPH_{-1}]^{4-}$		3.22 ± 0.05	1.93 ± 0.12
	P = Glycylglycine		
$[MAPH_2]^-$	36.04 ± 0.05		
$[MAPH]^{2-}$	30.56 ± 0.02	24.25 ± 0.01	23.93 ± 0.02
$[MAP]^{3-}$	22.36 ± 0.03	15.01 ± 0.01	14.37 ± 0.02
$[MAPH_{-1}]^{4-}$		3.12 ± 0.03	2.66 ± 0.05

Table 8. Overall Formation Constants of Divalent Metal Ion (M)−Ferulic Acid (A)−Glycine Peptide (P) Complexes at 298.15 K and 0.15 mol·dm^{-3} $NaNO_3$

species	log β_{pqrs} ± SD Cu(II)	Ni(II)	Co(II)
	P = Glycine		
[MAPH]	19.64 ± 0.06	17.09 ± 0.08	
$[MAP]^-$	13.11 ± 0.02	9.47 ± 0.02	8.31 ± 0.04
	P = Glycylglycine		
[MAPH]			15.43 ± 0.04
$[MAP]^-$	13.81 ± 0.02	8.74 ± 0.06	7.14 ± 0.04
$[MAPH_{-1}]^{2-}$	6.58 ± 0.03	−0.03 ± 0.04	−1.55 ± 0.04
	P = Glycylglycine		
$[MAP]^-$	11.90 ± 0.02	7.95 ± 0.04	6.76 ± 0.07
$[MAPH_{-1}]^{2-}$	5.86 ± 0.01	−1.07 ± 0.12	−1.88 ± 0.08
$[MAPH_{-2}]^{3-}$	−2.00 ± 0.03	−9.06 ± 0.02	−10.47 ± 0.03

Table 9. Δ log K Values of Mixed Ligand Complexes at 298.15 K in Aqueous Solution with Ionic Strength 0.15 mol·dm^{-3} $NaNO_3$

metal ion	Δ log K GA−G	GA−GG	GA−GGG	FA−G	FA−GG	FA−GGG
Cu(II)	−5.03	−1.93	−1.99	−0.39	2.99	1.34
Ni(II)	−0.79	0.16	0.65	0.03	0.71	0.21
Co(II)	0.48	1.42	1.57	0.22	0.46	0.08

Co(II) in the binary systems, while it shows a less positive Δ log K value than those of Ni(II) and Co(II). This phenomenon can be explained based on the metal−ligand interaction behavior in binary and mixed ligand systems. If the interaction between metal ion and a particular ligand to form binary system is strong, then it becomes difficult for a secondary ligand to bind to the ligand−metal and form a mixed ligand system which results in the less positive value of Δ log K. In some systems (Cu−GA−G, Cu−GA−GG, Cu−GA−GGG, Ni−GA−G, and Cu−FA−G), Δ log K are negative. These negative values may be the result of the high stability of their binary complexes, reduction in the coordination sites of the metal ions, steric hindrance, and electrostatic effect.[44−46] For the FA-GG and FA-GGG systems, Δ log K values have a similar tendency as their corresponding binary complexes. It may occur due to the competition between ligands since FA, GG, and GGG show almost similar chelating abilities toward metal ions.

From the equilibrium constants obtained, the distributions of various complex species formed in electrolyte solutions were illustrated by HYSS 2009 program as a function of pH. The typical species distribution diagrams of Cu(II) complexes are supplied in Figures 3 and 4. These diagrams clearly show that complexes indeed formed in a certain pH range. For systems containing gallic acid (Figure 3), GA is to be a very strong chelator for Cu(II) and thus hinders the formation of a ternary complex between Cu(II), gallic acid, and amide deprotonated glycine peptides such as $[CuAPH_{-1}]$ and $[CuAPH_{-2}]$. For systems containing ferulic acid, since FA forms a slightly less stable complex with metal ions compared to glycine, the competition between these two ligands can be seen in Figure 4a where at approximately pH 8, the [CuFAG], $[CuFA_2]$, and $[CuG_2]$ complexes were formed at nearly the same amount, 37 %, 27 %, and 28 %, respectively.

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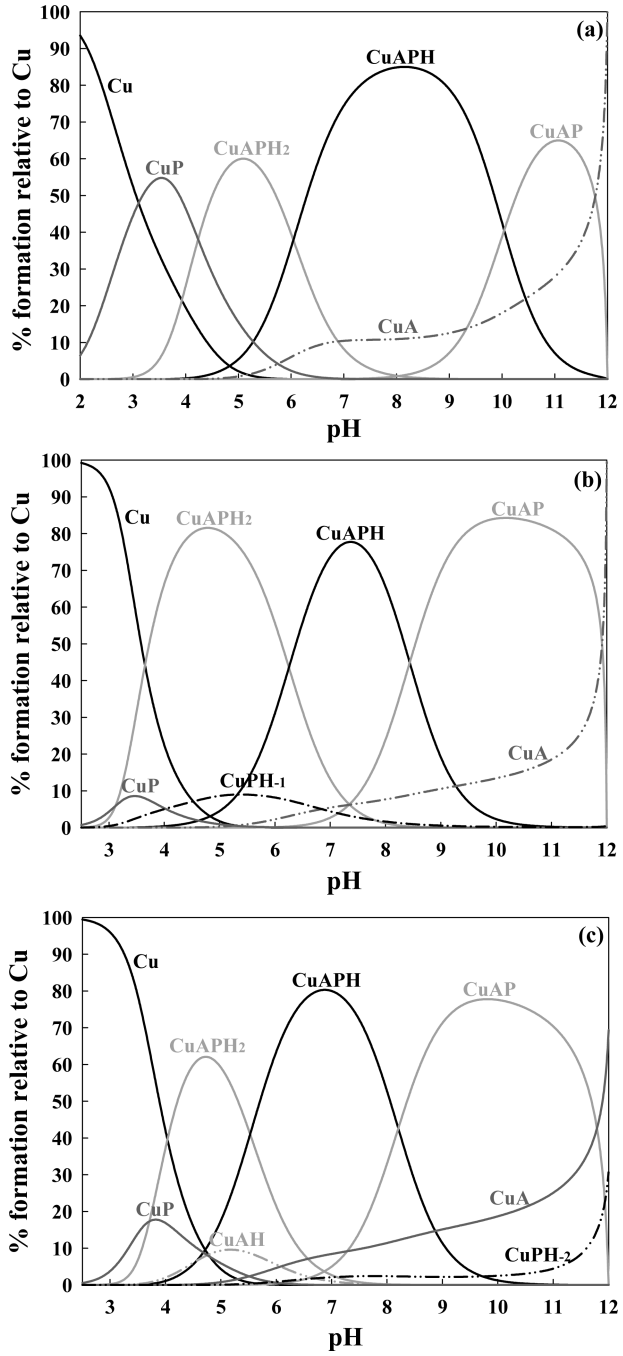

Figure 3. Species distribution diagrams of Cu(II) with (a) gallic acid (A)−glycine (P), (b) gallic acid (A)−glycylglycine (P), and (c) gallic acid (A)−glycylglycylglycine (P) at 298.15 K and 0.15 mol·dm^{-3} $NaNO_3$. Charges are omitted for simplicity.

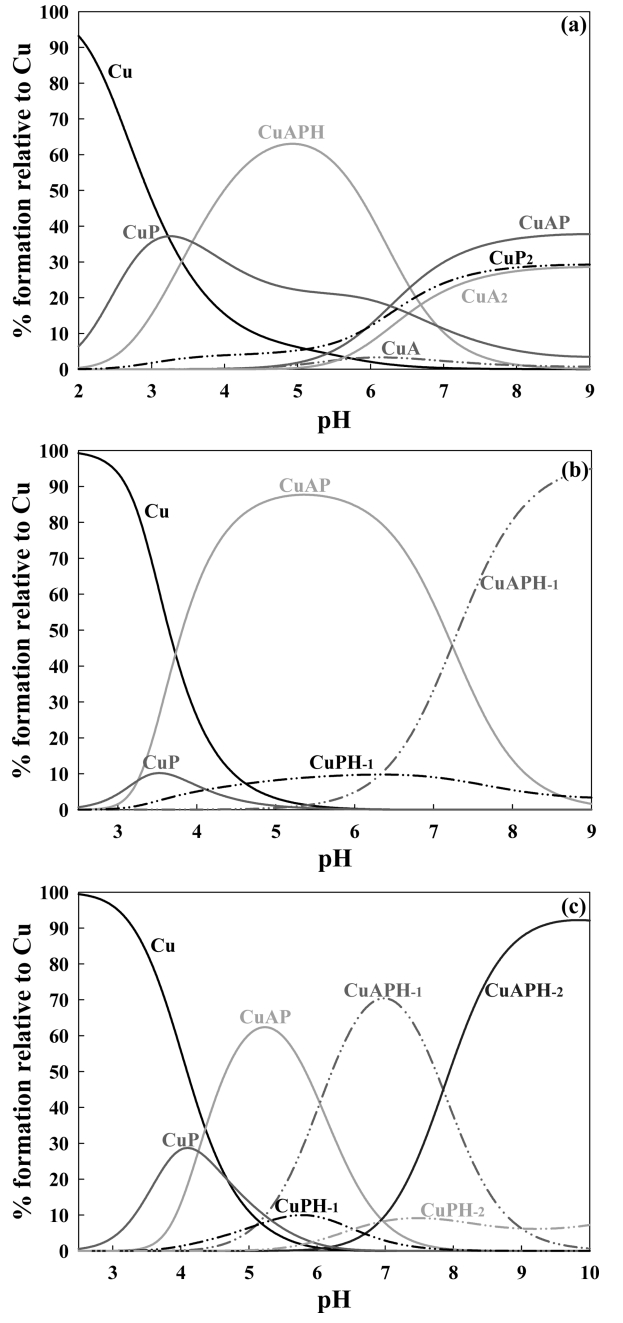

Figure 4. Species distribution diagrams of Cu(II) with (a) ferulic acid (A)−glycine (P), (b) ferulic acid (A)−glycylglycine (P), and (c) ferulic acid (A)−glycylglycylglycine (P) at 298.15 K and 0.15 mol·dm^{-3} $NaNO_3$. Charges are omitted for simplicity.

CONCLUSION

In this work, the interactions between divalent metal ions (Cu, Ni, and Co), phenolic acids (FA and GA), and glycine-contained peptides (G, GG, and GGG) were studied at 298.15 K and ionic strength I = 0.15 mol·dm^{-3} $NaNO_3$ by using the pH-potentiometry technique. The protonation constants and complex stability constants as overall formation constants (log β_{pqrs}) were obtained by the HYPERQUAD 2008 program. The contributions of deprotonated or undeptotonated amide peptide to the stability constant were studied. It was found that the amide nitrogen in peptide backbone enhanced the complex stability while it occurred in deprotonated form but may not affect in coordination with divalent metal ions while it occurred in underprotonated form. The stability constants of the complexes in both binary and mixed-ligand systems follow the Irving−Williams order: Cu(II) > Ni(II) > Co(II). The complexes were more stably formed in mixed ligand systems than the binary complexes. Moreover, in the mixed ligand system containing gallic acid, the complex is more stable than that with ferulic acid.

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Notes

The authors declare no competing financial interest.

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