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Investigation of copper(II) complexation by glycylglycine using isothermal titration calorimetry

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Abstract Isothermal titration calorimetry (ITC) and potentiometric titration methods have been used to study the process of proton transfer in the copper(II) ion-glycylglycine reaction. The stoichiometry, conditional stability constants, and thermodynamic parameters (ΔG, ΔH, and ΔS) for the complexation reaction were determined using the ITC method. The measurements were carried out at 298.15 K in solutions with a pH of 6 and the ionic strength maintained with 100 mM $NaClO_4$. Carrying out the measurements in buffer solutions of equal pH but different enthalpies of ionization of its components (Mes, Pipes, Cacodylate) enabled determination of the enthalpy of complex formation, independent of the enthalpy of buffer ionization. The number of protons released by glycylglycine on account of complexation of the copper(II) ions was determined from calorimetric and potentiometric measurements.

Keywords Cu(II)–glycylglycine complex · Proton exchange · Thermodynamic parameters · Isothermal titration calorimetry

Introduction

Owing to their catalytic properties, among others, copper(II) complexes with amino acids, peptides, and proteins have attracted the attention of many research teams [1–6]. Peptides have been known to act as chelating agents. Their complex-forming capacity has been utilized in medicine for treatment of Wilson's disease and removing excess copper from the liver and prevention of its accumulation [7–9].

Dipeptides devoid of electron-donating atoms in their side chains usually act as tridentate ligands over a pH range 4.0–7.0. Due to the formation of five-member chelate rings, thermodynamically stable copper(II) complexes are formed. X-ray studies have shown that the metal ions are co-ordinated through the oxygen atom of the carboxylic group, the nitrogen atom of the peptide bond, and that of the amino group [10–12]. This mode of co-ordination of the copper(II) ions has also been reported in aqueous solutions [13–15].

When the copper(II) ions bind to the glycylglycine ligand (GlyGly) they cause weakening of the hydrogen–nitrogen peptide bond thus promoting dissociation of the proton. Deprotonation of the peptide bond does not take place when the hydrogen atom of the bond is substituted, for instance with a methyl group, as is the case with glycylsarcosine (glycyl-*N*-methylglycine) [14]. Binding of copper(II) ions to peptides or proteins results in the change of their conformation. Furthermore, splitting off the proton during binding of the copper(II) ion is likely to trigger the change in the protonation degree of side chains of an amino acid. This would result in an alteration of its acid–base properties which, in turn, can affect its biological functions.

In order to describe the interaction of GlyGly with copper(II) ions and to gain knowledge of the mechanism of formation of a biologically relevant complex, calorimetric measurements were conducted. Isothermal titration calorimetry and potentiometric measurements were the methods used. In this article, a thermodynamic study of the copper(II) ion–GlyGly interaction is presented.

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Experimental

Materials

All reagents: $Cu(NO_3)_2{\cdot}2.5H_2O$, glycylglycine (≥99.5%), $NaClO_4{\cdot}H_2O$, Cacodylate (Cacodylic acid sodium salt trihydrate), Pipes (1,4-Piperazinediethanesulfonic acid), Mes 2-(*N*-Morpholino)ethanesulfonic acid) were purchased from Aldrich Chemical Corp. All compounds were used without further purification.

Isothermal titration calorimetry (ITC)

All ITC experiments were run at 298.15 K using an AutoITC isothermal titration calorimeter (MicroCal Inc., Northampton, USA). All details for the measuring devices and the experimental setup are described in [16, 17]. The experiment consisted of injecting 10.02 μL (29 injections, 2 μL for the first injection only) of a *ca* 3.5 mM solution of appropriate Cu^{2+} salt into the reaction cell initially containing buffered solution of a *ca* 0.33 mM glycylglycine, GlyGly (ionic strength $I = 100$ mM $NaClO_4$). A background titration was performed using identical titrant with the buffer solution placed in the sample cell. The result was subtracted from each experimental titration to account for the heat of dilution. The equilibrium binding constant K_{obs}, binding enthalpy ΔH_{obs}, and reaction stoichiometry n were obtained from ITC experiment by fitting binding isotherms, using nonlinear least-squares procedures, to a model that assumes a single set of identical binding sites. From these experimentally determined parameters, the free energy of binding (ΔG) and the entropy change (ΔS) were determined using the Gibbs equation: $\Delta G_{obs} = -RT\ln K_{obs.}$.

Potentiometric measurements

Potentiometric titrations were performed using an automatic titratory Cerko Lab® System with a 1.0-mL Hamilton's syringe interfaced with an IBM computer. The titrations were performed automatically by means of a suitable program Cerko Lab System ver. 3.OS-Expert. A constant-speed magnetic stirring was applied throughout. The temperature of the titration cell was kept at 298.15 K by means of a Lauda E100 circulation thermostat. The pH combined electrode was used (Schott). The electrode was calibrated according to IUPAC recommendation [18]. All the solutions were prepared directly before measurements. Aliquots (2.0 mL) of sample solutions, containing (*i*) glycylglycine and HCl in the 1:1.76 mol ratio and (*ii*) glycylglycine, HCl, and Cu^{2+} in the 1:1.76:1 mol ratio, were potentiometrically titrated with a standardized NaOH solution over the pH range 2.5–12.0.

Results and discussion

Table 1 summarizes stability constants K_{obs} determined by the ITC technique, the stoichiometry (Cu^{2+}/GlyGly), and thermodynamic quantities (ΔG, ΔH, ΔS) for the reaction of the copper(II) ions with GlyGly at 298.15 K, in Cacodylate, Pipes, and Mes buffer solutions with a pH of 6.

In solutions of pH 6, copper(II) ions form 1:1 complexes with GlyGly. A crystallographic examination has shown that the stoichiometry is also retained in the solid state [12]. Except for GlyGly, the co-ordination sphere of the metal also accommodates two water molecules to form a slightly distorted square pyramidal geometry. In aqueous solution, there are five water molecules in the co-ordination sphere of the copper(II) ion [19–21]. The positive values of the enthalpy of the complexation reaction, ΔH_{obs}, indicate that the positive endothermic effect due to dehydration of the ion ($\Delta H_{dehyd} > 0$) is not overcompensated by an exothermic effect due to the formation of new metal–ligand bonds ($\Delta H_{bind} < 0$) (Table 1). Consequently, thermodynamic stability of the complex depends on the entropy change. The positive value of the change is associated with the chelating effect resulting in the formation of two five-member rings (Scheme 1).

The ΔH_{obs} determined from the ITC experiment depends on the nature of the buffer solution in which the measurement was carried out (Table 1). In Fig. 1, the binding isotherms are shown, obtained in buffer solutions of different ionization enthalpies (Mes, Pipes, Cacodylate) with a pH of 6, at 298.15 K and the ionic strength $I = 100$ mM ($NaClO_4$).

The observed enthalpy of binding, ΔH_{obs}, is the sum of all energetic effects accompanying formation of the Cu–GlyGly complex. For this reason one should consider several factors which have influence on the binding thermodynamics: (*i*) the ionization enthalpy of the buffer, (*ii*) the enthalpy of proton–ligand dissociation, and (*iii*) the formation enthalpy of metal–buffer species. ΔH_{obs} increases inversely, proportionally to the ionization enthalpy of the buffer. Energetic input to ΔH_{obs} obtained from the ITC measurement is generated by proton exchange. This is an exchange between the ligand and a component of the buffer during complex formation. To determine the enthalpy change independent of the enthalpy of buffer ionization as well as the number of protons split off the GlyGly ligand, the following equation has been utilized [22–24]:

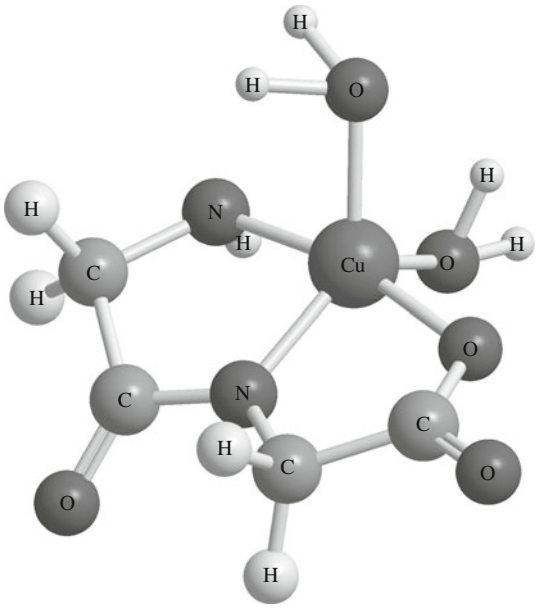
$$\Delta H_{obs} = \Delta H^{o}_{ML} - \Delta H^{o}_{ML} + \Delta n(\Delta H^{o}_{HB} - \Delta H^{o}_{HL}) \quad (1)$$

where ΔH^{o}_{ML} and ΔH^{o}_{MB} denote the enthalpy of metal–ligand complex formation and the enthalpy of metal–buffer dissociation species, respectively; ΔH^{o}_{HB} and ΔH^{o}_{HL} denote the enthalpy of buffer ionization and the enthalpy of proton–ligand dissociation, respectively; Δn is the number of protons exchanged during binding.

Table 1 Thermodynamic quantities of Cu^{2+} binding to GlyGly in the buffer solutions with a pH of 6, at 298.15 K and at an ionic strength $I = 100$ mM ($NaClO_4$)

	Mes	Pipes	Cacodylate
Stoichiometry	1.02 (0.01)	1.10 (0.01)	1.08 (0.01)
K_{obs}/M^{-1}	7.23 (0.02) × 10^4	4.55 (0.02) × 10^4	6.61 (0.21) × 10^4
ΔH_{obs}/kcal mol^{-1}	3.52 (0.01)	4.70 (0.02)	9.50 (0.05)
$T\Delta S$/kcal mol^{-1}	10.16	11.03	16.08
ΔG/kcal mol^{-1}	−6.629 (0.002)	−6.354 (0.003)	−6.576 (0.019)

In *parentheses*, the weighted mean standard deviation is given



Scheme 1 The coordination mode of copper(II) ion to glycylglycine anion

The relationship between ΔH_{obs} and ΔH^{o}_{HB} in buffer solutions with a pH of 6 is presented in Fig. 2. The ionization energies of the buffers used in this study are −0.71, 2.68, and 3.54 kcal mol^{-1} for Cacodylate, Pipes, and Mes, respectively [25]. The enthalpy of the reaction independent from the enthalpy of buffer ionization as calculated from Eq. 1 is 8.473 ± 0.096 kcal mol^{-1}.The decreasing ΔH_{obs} value with increasing buffer ionization enthalpy ($\Delta n < 0$) shows that during complexation of the Cu^{2+} ions, 1.398 ± 0.029 (±standard error) moles of protons are lost by one mole of the ligand [26]. For this reason, endothermic effects due to Cu^{2+}/GlyGly interaction in buffer solutions of positive ionization enthalpies (Mes, Pipes) are reduced by the energy released during proton binding to a buffer component ($\Delta H < 0$) (Fig. 1). With the Cacodylate buffer of negative ionization enthalpy (−0.71 kcal mol^{-1}), the proton transfer during complexation of the Cu^{2+} ions results in an increase in ΔH_{obs}.

In addition, potentiometric measurements were carried out to verify the number of protons released during the complexation, as determined by the ITC method. To do this, a solution containing GlyGly and HCl in the mole ratio of 1:1.76 (solution 1) and another—containing Cu^{2+}, GlyGly, and HCl in the mole ratio of 1:1:1.76 (solution 2) were titrated with a standardized NaOH solution. A relationship between the pH and the c_{NaOH}/c_{GlyGly} ratio is presented in Fig. 3. The difference between the number of moles of NaOH, expended for neutralization of solutions 1 and 2 up to a pH of 6, corresponds to the number of protons lost by GlyGly upon complexation of the copper(II) ions.

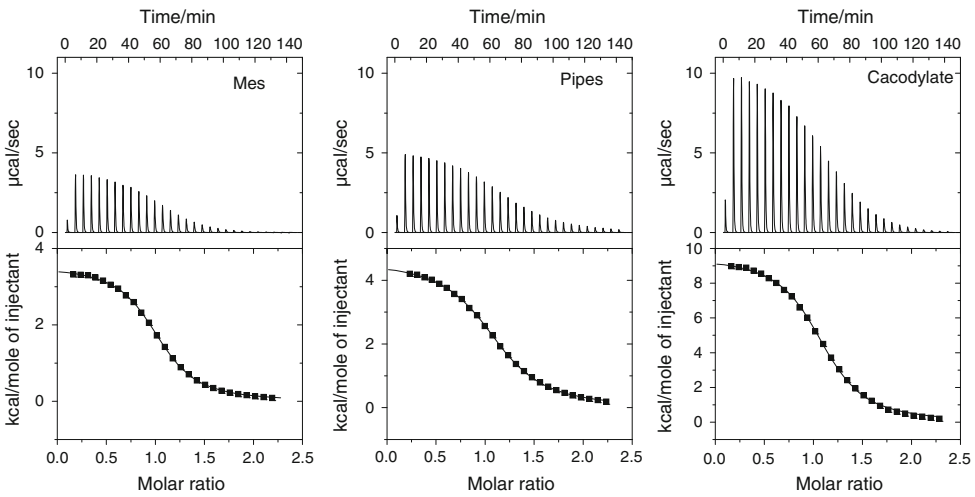


Fig. 1 Calorimetric titration isotherms of the binding interaction between Cu^{2+} and GlyGly in buffers with different ionization enthalpies (Mes, Pipes, Cacodylate, 20 mM each) of a pH of 6, at 298.15 K and at an ionic strength $I = 100$ mM ($NaClO_4$)

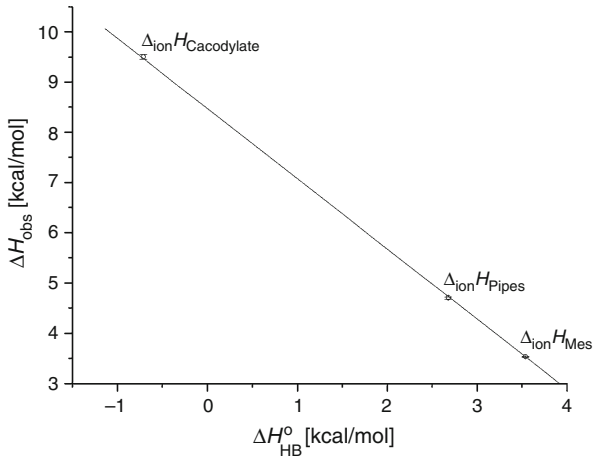


Fig. 2 Plot of ΔH_{obs} against ΔH^{o}_{HB} for the Cu^{2+}/GlyGly interaction in 10 mM Cacodylate, Pipes, and Mes, at a pH of 6, at 298.15 K

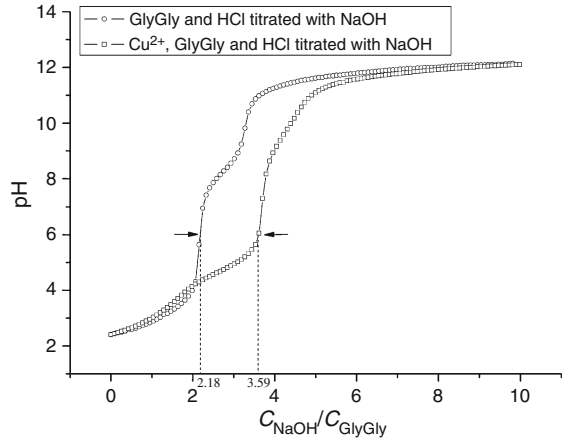


Fig. 3 Plot of the pH versus c_{NaOH}/c_{GlyGly} mole ratio during potentiometric titration of the GlyGly–HCl (*circle*) and Cu^{2+}–GlyGly–HCl (*square*) solutions with the NaOH solution

The number of protons determined in this way is 1.41 (c_{NaOH}/c_{GlyGly} {solution 2, pH 6} − c_{NaOH}/c_{GlyGly} {solution 1, pH 6}) (Fig. 3). This number is compatible, within the experimental error, with the number of protons determined by the calorimetric method.

Although literature data show that the interaction of Cu^{2+} with Mes is negligible and small [27], the interactions of Pipes with Cu^{2+} [28] and Cacodylate with some metal ions, e.g., Zn^{2+} were reported [29]. The differences in entropies (Table 1) almost compensate changes in enthalpy. This finding supports the idea that buffer molecules may be involved in Cu^{2+} coordination. For this reason the metal–buffer interactions should be taken under consideration during analysis of the ITC data. Assuming that the Cu^{2+} ion forms a 1:1 complex with the buffer (B) as well as taking into account three protonation states of GlyGly ligand (GGH^{+}, GG, GG^{-}), the individual equilibria that contribute to the overall equilibrium, as well as the coefficients that indicate the percentage of the Cu^{2+} and GlyGly species in solution under experimental conditions, are presented in Table 2.

Table 2 Individual equilibria that contribute to the overall equilibrium for the formation of Cu–GlyGly complex

	Reaction[a]	Coefficient	ΔH[b]
1	$CuB^{2+} = Cu^{2+} + B$	α_{CuB}	$-\Delta H^{o}_{CuB}$
2	$GGH^{+} = GG^{-} + 2H^{+}$	α_{GGH}	$-\Delta H^{o}_{GGH}$
3	$GG = GG^{-} + H^{+}$	α_{GG}	$-\Delta H^{o}_{GG}$
4	$Cu^{2+} + GG^{-} = CuGG + H^{+}$	1	$-\Delta H^{o}_{CuGG}$
5	$B + H^{+} = HB^{+}$	$1 + 2\alpha_{GGH} + \alpha_{GG}$	$-\Delta H^{o}_{HB}$

[a] Equilibria are written in the direction that the reaction occurs (1–3 are dissociations, 4 and 5 are associations)

[b] ΔH^{o} values are for association reactions

The overall reaction for the formation of Cu–GlyGly complex is given by Eq. 2.

$$\begin{aligned}(1-\alpha_{CuB})Cu^{2+} + \alpha_{CuB}CuB^{2+} + \alpha_{GGH}GGH^{+} + \alpha_{GG}GG \\ + (1-\alpha_{GGH}-\alpha_{GG})GG^{-} + (1+2\alpha_{GGH}+\alpha_{GG} \\ - \alpha_{CuB})B \\ = \ CuGG \ + (1+2\alpha_{GGH}+\alpha_{GG})BH^{+}\end{aligned} \tag{2}$$

Coefficients α_{CuB}, α_{GGH}, α_{GG} depend on a pH of solution, proton–ligand association constants K_{GGH}, K_{GG}, and Cu^{2+}-buffer association constant K_{CuB}. They are represented by the Eqs. 3–5:

$$\alpha_{CuB} = \frac{K_{CuB}[B]}{1 + K_{CuB}[B]} \tag{3}$$

$$\alpha_{GGH} = \frac{K_{GGH}K_{GG}[H^{+}]^{2}}{1 + K_{GG}[H^{+}] + K_{GGH}K_{GG}[H^{+}]^{2}} \tag{4}$$

$$\alpha_{GG} = \frac{K_{GG}[H^{+}]}{1 + K_{GG}[H^{+}] + K_{GGH}K_{GG}[H^{+}]^{2}} \tag{5}$$

In solution of a pH of 6 the value of α_{GGH} and α_{GG} coefficients equal 0.19 and 1.25×10^{-3}, respectively. The observed enthalpy of binding, ΔH_{obs}, can be expressed by Eq. 6, which is based on Hess's law:

$$\begin{aligned}\Delta H_{obs} = \ &-\alpha_{CuB}\Delta H^{o}_{CuB} - \alpha_{GGH}\Delta H^{o}_{GGH} - \alpha_{GG}\Delta H^{o}_{GG} \\ &+ \Delta H^{o}_{CuGG} + (1+2\alpha_{GGH}+\alpha_{GG})\Delta H^{o}_{HB}\end{aligned} \tag{6}$$

where $1 + 2\alpha_{GGH} + \alpha_{GG}$ corresponds to the number of protons transferred. From the Eq. 6 pH and buffer-independent enthalpy of Cu–GlyGly complex formation (ΔH^{o}_{CuGG}) may be determined.

Conclusions

Based on the results of isothermal titration calorimetry, thermodynamic parameters (ΔG, ΔH, and ΔS) have been

determined for the complexation reaction of the Cu^{2+} ions with glycylglycine, as well as the stoichiometry of the complex formed and its conditional stability constants in solution with a pH of 6, at 298.15 K. In addition, enthalpy of the complexation reaction independent of the enthalpy of buffer ionization was determined in solutions of equal pH but with different ionization enthalpies. The numerical value of the enthalpy is 8.473 ± 0.096 kcal mol^{-1}. On the basis of the relationship between the observed enthalpy of binding, ΔH_{obs}, and that of the buffer ionization, ΔH^{o}_{HB}, it was found that the number of moles of the protons lost by 1 mol of glycylglycine during complexation of the Cu^{2+} ions, at a pH of 6, is 1.398 ± 0.029. This result obtained by the ITC method was subsequently verified by the potentiometric titration technique. In order to determine pH and buffer-independent enthalpy of Cu–GlyGly complex formation (ΔH^{o}_{CuGG}), the values of proton–ligand dissociation enthalpies ($\Delta H^{o}_{GG,}$ ΔH^{o}_{GGH}) and the value of stability constant (K_{CuB}) as well as enthalpy of formation of metal-buffer complex(es) (ΔH^{o}_{CuB}) should be known.

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