Speciation Studies of Copper Complexes with L-Glutamic Acid by Using Diethylaminoethyl Sephadex A-25 Column

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A method to evaluate the complexing ability of copper(II) with L-glutamic acid was studied using a weak-base anion exchanger, diethylaminoethyl Sephadex A-25 (A-25). The species of copper(II) complex with L-glutamic acid were $\text{Cu}(\text{Glu})^0$ (1:1 complex) and $\text{Cu}(\text{Glu})_2^{2-}$ (1:2 complex). The 1:2 complex was retained on A-25 since this complex was anionic. The amount of copper retained on A-25 as a 1:2 complex and that which flowed out as free copper(II) ion and a 1:1 complex were determined by flame atomic absorption spectrometry. From the concentration of the 1:2 complex measured, the equations to calculate the stability constants (β_1 and β_2) were derived in terms of the equilibria of the solution; the stability constants obtained were in good agreement with values in the literature. The distribution curves of copper species at various pH were also in good agreement with curves simulated from the estimated stability constants.

Keywords Copper, L-glutamic acid, stability constants, diethylaminoethyl Sephadex A-25

Heavy-metal ions in natural water exist in various form: for example, adsorbed on suspended matter and clay minerals, complexed with inorganic and organic matter, and as hydrate ions.¹⁻³ Clarifying these chemical forms is very important for biological and environmental aspects. Studies concerning the speciation of metal ions in natural water have been performed using ion-selective electrodes (ISE) that respond to the activity of free metal ions^{4,5}, anodic stripping voltammetry (ASV) that measure free metal ions and labile species⁶, as well as cation exchange methods.^{7,8} However, the ISE method is not sufficiently sensitive to measure trace substances in natural water; the ASV method has a problem that the complex would dissociate in the diffusion layer during electrolysis.⁹

Previously, Hiraide *et al.* reported that the macroreticular weak-base anion exchanger, diethylaminoethyl Sephadex A-25 (A-25), could be used to measure the species of heavy-metal ions (Fe, Cu, Cd, Pb) binding with humic substances and other anionic ligands in river water.¹⁰⁻¹² They evaluated the copper-complexing ability of humic substances using an A-25 column.¹³ We also reported on a method to quantitatively evaluate the copper-complexing ability of humic acids in peat soil by A-25.¹⁴

On the other hand, it was known that there were many other organic ligands than humic substances in natural water: for example, amino acids and fatty acids.¹⁻³ These materials were also important in influencing the heavy-metal complexing capacity of natural water as well as humic substances. Especially, Uchiyama *et al.* reported that the amount of amino acids (free or bound form) correlated with the copper

complexing capacity in natural water.¹⁵⁻¹⁷

In the present work, a method for evaluating the complexing ability of amino acids in natural water was investigated using A-25. L-Glutamic acid was selected as an example of amino acid in natural water, because L-glutamic acid is widely distributed in nature. The species of copper complex with L-glutamic acid were $\text{Cu}(\text{Glu})^0$ as a 1:1 complex and $\text{Cu}(\text{Glu})_2^{2-}$ as a 1:2 complex. It seemed that only the 1:2 complex was retained by ion exchange on A-25 since it had an anionic charge. The adsorption behavior of copper/L-glutamic acid complex on A-25 was investigated, and the stability constants (β_1 and β_2) of their complexes were estimated.

Experimental

Reagents and materials

L-Glutamic acid solution was prepared by dissolving L-glutamic acid (Wako Pure Chemical Industries, Ltd.) in a 0.01 M potassium hydroxide solution.

The standard copper solution (1 g/l) was prepared by dissolving electric copper (Mitsuwa Pure Chemicals, purity 99.999%) in concentrated nitric acid and diluting to a 0.01 M nitric acid solution.

The diethylaminoethyl Sephadex A-25 (Pharmacia LKB Biochemistry, Cl⁻ form) was washed by ultrasonic irradiation for 20 min in a 0.1 M hydrochloric acid solution and then washed with water. It was next swollen in a 0.05 M potassium chloride solution with an adjusted pH of 6–7 by a potassium hydroxide solution. Two milliliters of A-25 were packed into a column

which was washed with 50 ml of water and passed through a 20 ml of a buffer solution at the experimental pH.

The other reagents were of analytical reagent grade (Wako Pure Chemical Industries, Ltd.).

Apparatus and instruments

A polypropylene column of 40 mm×8 mm i.d. (Bio-Rad) was used. The copper concentration was measured by a HITACHI 170-50 atomic absorption spectrometer (AAS) with the PTFE funnel to the nebulizer needle. The absorbance of the copper was measured at 324.7 nm in the air/acetylene flame. A HORIBA M8L-type pH meter was used for the pH measurement.

Procedure

After the pH of a sample solution containing copper(II) and L-glutamic acid was adjusted, 15 ml of the sample solution was passed through the column at a flow rate of 5 ml/min. The A-25 column was then washed with water. The A-25 in the column was transferred to a centrifuge tube and 8 ml of 4 M nitric acid solution was added. The copper adsorbing on A-25 was desorbed by ultrasonic irradiation for 5 min. The solution and A-25 was separated by centrifugation for 3 min and the supernatant was transferred to a 50 ml beaker. Eight milliliters of 4 M nitric acid solution were again added to the centrifuge tube; the same procedure was then repeated. The supernatant introduced into a 50 ml beaker was dried up on a hot plate. The residue was decomposed by adding 0.5 ml of concentrated nitric acid and diluting to 10 ml. One hundred microliters of the aliquot was introduced into a PTFE funnel of AAS. The concentration of the 1:2 complex could be calculated by dividing the amount of copper on A-25 by the volume of the original sample solution. The effluent containing free copper and a 1:1 complex was enriched by heating on a hot plate and then diluted to 10 ml with a 0.2 M nitric acid solution. One hundred microliters of the aliquot was introduced into AAS.

Results and Discussion

Adsorption behavior of copper/L-glutamic acid on A-25 column

L-Glutamic acid forms a 1:1 or 1:2 complex with copper(II). At pH=6, the 1:2 complex would be mainly an anionic species. When the acetate buffer solution at pH 5.5 or 6 containing copper(II) ion was passed through the A-25 column, the copper was not retained on A-25 at all. But when L-glutamic acid was added into this solution, copper was retained on the A-25 column. It therefore was seemed that a 1:2 complex of copper/L-glutamate could be retained on A-25 by ion-exchange, but the cationic or neutral species (for example, free copper ion and a 1:1 complex) could not

be retained and flowed out under these conditions.

Effect of the flow rate of sample solution

In the case of studying the complexing equilibrium in a solution by ion-exchange methods, it is important that the complexing equilibrium in the solution does not change during ion-exchange. Therefore, the effect of the flow rate of the sample solution on the amount of copper retained on A-25 was investigated. At various ratios of the concentration of copper to L-glutamic acid, the amount of copper retained on A-25 was constant at flow rates within the range 1 to 20 ml/min. It therefore, appeared that the amount of copper retained on A-25 would be influenced by the ratio of the concentration of copper to L-glutamic acid in the solution irrespective of the flow rate. That is, these results obtained by an A-25 column method would reflect the complexing equilibria in the solution and the equilibria would not change during ion-exchange.

Estimation of the stability constants of copper(II)/L-glutamic acid

In order to estimate the copper complexing ability of L-glutamic acid by measuring the copper retained on A-25 as a 1:2 complex, the complexing equilibrium in the solution is treated as illustrated below. The complex formation of copper(II) (M) with L-glutamic acid (L) is written as

and

At this time, the stability constants (β_1 and β_2) are written as

$$\beta_1 = [ML]/([M][L]) \quad (3)$$

and

$$\beta_2 = [ML_2]/([M][L]^2) \quad (4)$$

From the mass balance of the solution,

$$[M_t] = [M] + [ML] + [ML_2] \quad (5)$$

where $[M_t]$ is the total copper concentration of the original solution. L-Glutamic acid is in association equilibria with protons in the solution. The total concentration of L-glutamic acid in the original solution (c_L) can be written as

$$c_L = [L] + [ML] + 2[ML_2] + [HL] + [H_2L] + [H_3L] \quad (6)$$

The side reaction coefficient for protonation of L-glutamic acid, $\alpha_{L(H)}$, is written as

$$\alpha_{L(H)} = ([L] + [HL] + [H_2L] + [H_3L]) / [L]$$

$$= 1 + K_{HL}[H] + K_{HL}K_{H2L}[H]^2 + K_{HL}K_{H2L}K_{H3L}[H]^3, \quad (7)$$

where K_{HL} , K_{H2L} and K_{H3L} are consecutive stability constants of L-glutamic acid with a proton. From the stability constants reported by Kortüm *et al.*¹⁸, and the concentration of protons measured by the glass electrode, $\alpha_{L(H)}$ could be calculated.

Moreover, Eq. (6) can be divided by $[L]$; from Eq. (7),

$$c_L/[L] = ([ML] + 2[ML_2]) / ([L] + \alpha_{L(H)}). \quad (8)$$

From a rearrangement of Eq. (8),

$$[L] = (c_L - ([ML] + 2[ML_2])) / \alpha_{L(H)}. \quad (9)$$

Equation (5) is divided by $[M]$ and rearranged using Eqs. (3) and (4),

$$[M_i]/[M] = 1 + [ML]/[M] + [ML_2]/[M]$$

$$= 1 + \beta_1[L] + \beta_2[L]^2. \quad (10)$$

Further from a rearrangement of Eq. (10)

$$([M_i]/[M] - 1) / [L] = \beta_1 + \beta_2[L]. \quad (11)$$

Equation (9) is put into Eq. (11),

$$([M_i]/[M] - 1) \alpha_{L(H)} / (c_L - ([ML] + 2[ML_2]))$$

$$= \beta_1 + \beta_2 (c_L - ([ML] + 2[ML_2])) / \alpha_{L(H)}. \quad (12)$$

When the c_L value was larger than $[M_i]$, the following equation could be assumed:

$$[ML_2] \gg [ML]. \quad (13)$$

The validity of this assumption is discussed later. From Eq. (12) and Eq. (13) can be simplified as

$$([M_i]/[M] - 1) \alpha_{L(H)}^2 / (c_L - 2[ML_2])$$

$$= \alpha_{L(H)} \beta_1 + \beta_2 (c_L - 2[ML_2]). \quad (14)$$

Equation (14) shows that there was a linear relationship between $c_L - 2[ML_2]$ and $([M_i]/[M] - 1) / (c_L - 2[M_i])$, and that the β_1 and β_2 values could be estimated by the intercept and the slope of this line, respectively. $[ML_2]$ could be obtained by measuring the copper on A-25 by AAS, and the $[M]$ and $c_L - 2[ML_2]$ values could be calculated from Eqs. (5) and (6). Under the conditions that $c_L = 50 \mu M$ and $pH = 6$, a $c_L - 2[ML_2]$ vs. $([M_i]/[M] - 1) / (c_L - 2[M_i])$ plot was drawn (Fig. 1). The stability constants estimated by the intercept and the slope of this line were as follows: $\log \beta_1 = 6.0 \pm 0.3$, $\log \beta_2 = 15.2 \pm 0.1$ ($n=3$). The value of $\log \beta_2$ was in good agreement with the value reported by Norman *et al.*¹⁹

Fig. 1 Calculation of stability constants. $[M_i] = 0 - 15.9 \mu M$, $c_L = 50 \mu M$, $pH = 6$.

By using the β_1 and β_2 values obtained, the concentration of copper species $[M]$, $[ML]$ and $[ML_2]$ were simulated by computer; these values were then compared with the experimental values. The conditional stability constants (β'_1 and β'_2) are written as

$$\beta'_1 = \beta_1 / \alpha_{L(H)} \quad (15)$$

and

$$\beta'_2 = \beta_2 / \alpha_{L(H)}. \quad (16)$$

The concentration of copper species $[M]$, $[ML]$ and $[ML_2]$ could be calculated according to

$$[M] = [M_i] / (1 + \beta_1[L] + \beta_2[L]^2) \quad (17)$$

$$[ML] = [M_i] \beta_1 [L] / (1 + \beta_1[L] + \beta_2[L]^2) \quad (18)$$

and

$$[ML_2] = [M_i] \beta_2 [L]^2 / (1 + \beta_1[L] + \beta_2[L]^2). \quad (19)$$

The β'_1 and β'_2 values could be calculated from the $\alpha_{L(H)}$ value of L-glutamic acid at $pH = 6$. The simulation curve of $[ML_2]$ vs. $[M_i]$ is given in Fig. 2. This curve is in good agreement with the experimentally measured $[ML_2]$. Moreover, the simulation curves of $[M_i]$ vs. $[M]$ and $[M_i]$ vs. $[ML]$ are also given in Fig. 2. Since $[ML]$ was less than 3% of $[ML_2]$ over the range 0 to $16 \mu M$ $[M_i]$, it was confirmed that the assumption $[ML] \ll [ML_2]$ at Eq. (13) was appropriate.

Stability constants of various c_L

If the c_L values were known, stability constants β_1 and β_2 could be estimated by only measuring $[ML_2]$ at a certain $[M_i]$ and pH . Equation (9) was substituted in Eq. (4) and from an approximation of Eq. (13),

$$\beta_2 = \alpha_{L(H)}^2 [ML_2] / ([M] (c_L - 2[ML_2]))^2. \quad (20)$$

Fig. 2 Concentration of the copper species with L-glutamic acid (L). The simulation curves: (a), [M]; (b), [ML₂]; (c), [ML]. O, [ML₂] measured. [M_i]=0–15.9 μM, c_L=50 μM, pH=6.0.

Table 1 Stability constants of copper/L-glutamic acid complexes for various c_L

c _L /μM	35	40	50	75	100
log β ₁	5.8	6.0	6.2	5.8	6.1
log β ₂	15	15	15	15	15

[M_i]=15.9 μM, pH=6.

From the simultaneous equation between Eq. (14) and (17), the β₁ and β₂ values could be estimated. They are shown in Table 1. The values of β₁ and β₂ were in good agreement with the values estimated by the plot in Fig. 1.

Copper(II) species in sample solutions at various pH

The concentrations of the copper species in a solution containing L-glutamic acid at various pH were calculated using the β₁ and β₂ values; the distribution curves are given in Fig. 3. [ML₂] and the copper concentration in experimentally measured effluent are also given in Fig. 3 (O or ● in Fig. 3) and are in good agreement with the calculated distribution curves. Under the condition of more excess ligand concentration than [M_i], [ML] hardly formed and copper was present almost completely as [ML₂]. It thus seemed that assuming Eq. (13) was appropriate. From these results it was confirmed that the only 1:2 complex, [ML₂], was quantitatively retained on the A-25 column. It was therefore suggested that the A-25 column could be used to quantitatively evaluate the copper complexing ability of an organic ligand, such as an amino acid in natural water.

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Fig. 3 Distribution curves of copper species at various pH. The simulation curves: (a), [M]; (b), [ML₂]; (c), [ML]. O, copper on A-25; ●, copper in effluent, measurement values. [M_i]=4.0 μM, c_L=50 μM.

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