The Extraction of Copper(I) Ions with Heterocyclic Bidentate Amines in the Presence of Glutathione

Kotaro MORITA, Kazuki NAKANO and Hisanori IMURA*

Division of Material Sciences, Graduate School of Natural Science and Technology, Kanazawa University,

Kakuma, Kanazawa 920–1192, Japan

Copper(I) ions are generally unstable in aqueous solution and readily disproportionate to copper(II) and copper(0).   In this work, copper(I) is formed and stabilized under aerobic conditions by the addition of glutathione (GSH) which acts as a reducing and complexing agent.   The extraction of Cu(I) with heterocyclic bidentate amines such as 2,9-dimethyl-1,10-phenanthroline (dmp), 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, and 2,2'-biquinoline has been studied in the presence of GSH.   The formation of the Cu(I)-GSH complex in the aqueous solution was confirmed by spectrophotometry under aerobic conditions. Time-course measurements of the absorbance indicated that the Cu(I)-GSH complex retained stability toward re-oxidation by air for at least 6 h in the presence of a 10-fold excess of GSH at pH 5-7.   The quantitative extraction of copper(I) was accomplished with 5.0×10^{-4} M dmp in chloroform or 1,2-dichloroethane in the presence of 5.0×10^{-4} M GSH and 0.10 M ClO_4^- at pH 3-6.   The extracted species was found to be $Cu(dmp)_2ClO_4$ by the substoichiometric extraction method.

1. Introduction

Copper widely occurs in biological and geological materials and is found in different oxidation states, typically 0, +1, and +2, which depend on the chemical environment of copper in the materials.   In biological systems, it is known that Cu(I) and Cu(II) exist and play an important role in redox and electron transport processes.

Since Cu(I) ions in aqueous solution is unstable and readily disproportionate to Cu(II) and Cu(0), any ligand that forms stable Cu(I) complexes is essential to prevent the disproportionation reaction.   In the previous studies concerning the extraction of Cu(I), Cu(II) was reduced to Cu(I) using reducing agents such as hydroxylamine hydrochloride and ascorbic acid in the presence of chloride [1], thiocyanate [2,3], and halides[4] as the designated ligand (X^-) and extracted with quaternary ammonium ions (Q^+) into 1,2-dichloroethane and chloroform as an ion pair, $Q^+ \cdot CuX_2^-$.   On the other hand, the well-known bidentate ligands, 1,10-phenanthroline [5], 2,9-dimethyl-1,10-phenanthroline [6,7], and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline [8], have been employed for the spectrophotometric determination of Cu(I) after the reduction of Cu(II) with hydroxylamine and extraction into alcohol solvents.   To the best of our knowledge, there is no study on the extraction equilibrium of Cu(I) with those ligands in spite of many spectrophotometric applications [9].   This was due to lack of knowledge of the stabilization of the Cu(I) species in not only the organic but also the aqueous phases in the equilibrium study.

In this paper, glutathione (GSH) was used as the reducing agent for preparation of the Cu(I) solution. It is expected that the Cu(I) ions formed in the aqueous solution are stabilized under aerobic conditions with GSH which acts as a reducing and complexing agent. The effect of GSH on the stabilization of the Cu(I) state was examined and compared with those of common reducing agents, hydroxylamine and cysteine [10]. The extraction of the Cu(I) ion was studied with several heterocyclic bidentate amines such as 2,9-dimethyl-1,10-phenanthroline (dmp), 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (dmdpp), and 2,2'-biquinoline (bq) in the presence of GSH. The formation of the Cu(I)-GSH complex in the aqueous solution and the extraction equilibrium of Cu(I) in this system was studied. The composition of the extracted species was confirmed by the substoichiometric extraction of Cu(I) with a substoichiometric amount of dmp in the presence of an excess of a counter ion, ClO_4^-, and *vice versa*.

2. Experimental

2.1 Reagents

GSH (Tokyo Chemical Industry, $\geq$ 97.0 %), dmp (Sigma Aldrich, 99 %), dmdpp (Tokyo Chemical Industry, $\geq$ 99.0 %), and bq (Tokyo Chemical Industry, $\geq$ 99.0 %) were used as received. Prior to use, 1,2-dichloroethane (DCE, Sigma Aldrich, $\geq$ 99.5 %) was washed with 3 M sulfuric acid, 3 M NaOH, and water. Chloroform (Nacalai Tesque, 99 %) was washed three times with

Figure 1. Chemical structure of monoprotonated glutathione (GS^{2-})

water to remove ethanol added as a stabilizer. 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, Dojindo, $\geq$ 99 %) and 2-morpholinoethanesulfonic acid monohydrate (MES, Dojindo, $\geq$ 99%) were used to prepare buffer solutions. All other reagents were of commercially available analytical grade and were used without further purification. Water was deionized ($\geq$ 18.0 MΩ cm specific resistance) with an Elix UV Water Purification System and a Synergy UV system (Millipore Corp., Bedford, MA).

2.2 Apparatus

A Hitachi model Z-6100 polarized Zeeman atomic absorption spectrophotometer (FAAS) was used for the determination of Cu in the aqueous solutions. A Horiba model F-52 pH meter equipped with a combination glass electrode was used to determine the pH value. Absorption spectra were measured with a JASCO V-570 spectrophotometer using 1 cm quartz cuvettes. The time-course measurements of the absorbance were conducted with a Shimadzu UV-160 spectrophotometer.

2.3 Preparation of copper (I, II) solutions

Cu(II) stock solutions were prepared by dissolving Cu(II) chloride in 0.1 M hydrochloric acid or Cu(II) sulfate pentahydrate in 0.05 M sulfuric acid. Aqueous solutions of Cu(I) were prepared by adding a reducing agent such as GSH, cysteine, and hydroxylamine to the Cu(II) solutions. The reducing agents were dissolved in water just before use.

2.4 Time-course measurement of the absorbance of Cu(I) in the aqueous solution

Following measurements of change of the absorbance were carried out after 1 min of the addition of the reducing agent to the Cu(II) solution. Absorption spectra were recorded for 2 h at regular intervals and the absorbance changes at absorption maxima were also recorded for 12 h. Several reducing conditions were examined at different molar ratios of the reducing agent to copper ([reducing agent]/[Cu])
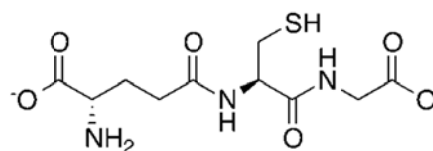
ranging from 1 to 10 for GSH.

2.5 Extraction procedures

An aqueous solution containing 1.0×10^{-7}-2.0×10^{-4} M Cu, 5.0×10^{-4} M GSH, cysteine, or hydroxylamine, and 1.0×10^{-1} M $(H,Na)ClO_4$ was shaken with an equal volume of an organic solvent containing 1.2×10^{-4} - 5.0×10^{-3} M dmp, dmdpp, or bq for 5-240 min.   After phase separation, the pH of the aqueous phase was measured, and the concentration of Cu in the aqueous phase was determined by FAAS.   An aliquot of the organic phase was shaken with 1.0 M nitric acid for 1 h to strip Cu into the aqueous solution, which was then analyzed by FAAS.   The distribution ratio (D) and the percent extraction (%E) of Cu were calculated from the Cu concentrations in the organic and the aqueous phase.

3. Results and Discussion

3.1 Reduction of Cu(II) with reducing agents in aqueous solution

The reduction of Cu(II) to Cu(I) was monitored by UV spectra upon the addition of reducing agents at pH 1-10. Figure 2 shows the UV spectra of the solutions obtained by adding a 10-times excess of the reducing agents to the Cu(II) solution at pH 7.4 (for concentrations, see the figure legend).   In the absence of reducing agents, Cu(II) (as chloride) exhibits a broad absorption band with a maximum at 280 nm. New absorption bands with a peak at 260 nm and a shoulder at 300 nm were recognized in the presence of both GSH and cysteine, whereas hydroxylamine gave a quite broad spectrum but no maximum at 280 nm.
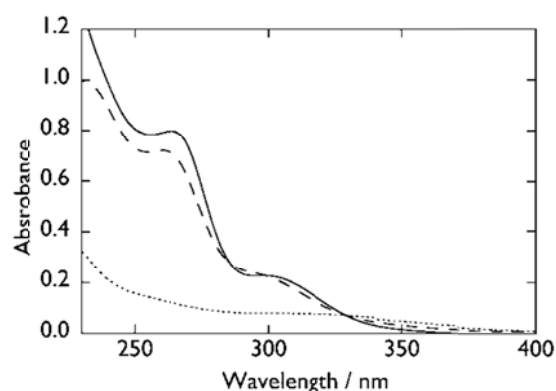

Figure 2.   UV spectra of Cu(I) in the presence of different reducing agents.   [Cu] = 1.0×10^{-4} M, [reducing agent] = 1.0×10^{-3} M, [HEPES] = 0.1 M, pH 7.4.   (solid line) GSH, (dashed line) cysteine, (dotted line) hydroxylamine.

The absorption bands at 260 nm and 300 nm in GSH and cysteine were attributed to complex formation of the Cu(I) ion with the thiol compounds [10,11].   The values of the half-cell potential of the GSH/glutathione disulfide(GSSG) and cysteine/cystine redox system were reported to be −0.262 and −0.245 V at pH 7.0 respectively [12], which were low enough to reduce the Cu(II) ion (0.153 V) in the aqueous solution.   These observations support the following redox reaction proposed in the literature [13],

$$2Cu^{2+} + 6GSH^- \rightarrow 2Cu^I(GS)_2^{3-} + GSSG^{2-} + 6H^+,$$

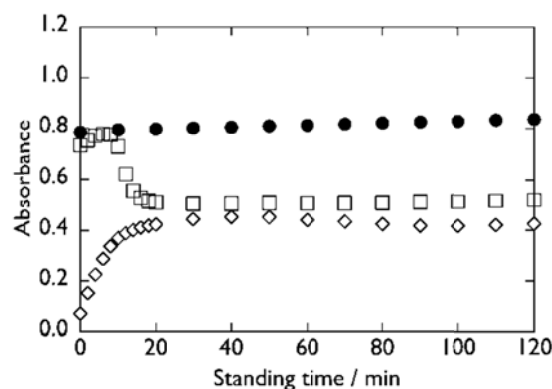where GS^{2-} stands for monoprotonated glutathione.

Figure 3.   Effect of standing time on the absorbance of the Cu solution after the addition of different reducing agents.   [Cu] = 1.0×10^{-4} M, [reducing agent] = 1.0×10^{-3} M, [HEPES] = 0.1 M, pH 7.4.   (●) Cu+GSH at 260 nm, (□) Cu+cysteine at 260 nm, (◊) Cu+hydroxylamine at 280 nm.

Figure 3 shows the time dependency of the absorbances at 260 nm in the presence of GSH and

cysteine, and at 280 nm in hydroxylamine.   In the hydroxylamine case, an increase in the absorbance at 280 nm is observed within 20 min and is ascribed to the autoxidation of Cu(I) to Cu(II).   On the other hand, Cu(I)-cysteine shows a marked decrease in the absorbance at 260 nm after 10 min under aerobic conditions, *i.e.*, the decay of the Cu(I)-cysteine complex.   When the same experiment was performed with GSH, the absorbances at 260 nm and 300 nm were unchanged up to 6 h, indicating that the oxidation state of copper remains univalent for several hours even under aerobic conditions.   Consequently, GSH is an effective reducing and complexing agent to reduce Cu(II) to Cu(I) and for stabilization of the Cu(I) state.

All the data given above are consistent with the formation of Cu(I)-GSH complexes accompanied by the reduction of Cu(II) and with their continuous regeneration against the autoxidation of Cu(I) under aerobic conditions.   In accordance with the interpretation given above, the length of time preceding the decay of the complexes (indicated by the absorbance at 260 nm) is strictly related to the excess amount of GSH.   Indeed, the 260 nm absorbance of Cu(I)-GSH at pH 7.4 remains the same for 2, 4, and 6 h with [GSH/[Cu(II)] ratios of 5, 7, and 10, respectively.   This result leads to a convenient method for the preparation of the aqueous Cu(I) solution under aerobic conditions.   At high GSH concentrations, the presence of Cu(I) prepared in the aqueous phase lasts longer up to 6 h, at which time almost all the GSH was oxidized to GSSG in the reduction of Cu(II) and the autoxidation.   It is, however, recognized that the existence of Cu(I) is pH dependent.   In the lower pH region, the existence of Cu(I) was slightly increased up to 8 h at pH 5 or 6.   In contrast, the existence of Cu(I) was less than 1 h above pH 9 (figure not shown). It is suggested that the decrease in the Cu(I) presence at high pH is due to the dissociation of the thiol group of GSH ($pK_a = 9.43$ [13]) and the formation of GSSG.   Hence, the aqueous solution of Cu(I) for extraction was prepared at below pH 8.

3.2 Extraction behavior of Cu(I) in the presence of GSH

Extraction of Cu(I) with 5.0×10^{-4} M bidentate amines (L) such as dmp, dmdpp, and bq in DCE was studied in the presence of 5.0×10^{-4} M GSH and 0.1M (H, Na)ClO$_4$.   The Cu(II) solution was initially added to the aqueous phase just before shaking.   The extraction curves with these amines are shown in Figure 4.   High extractability was obtained with dmp and dmdpp but the extractability with bq was low.   The difference observed among these amines is primarily explained by the stability of the Cu(I)-L complexes; *i.e.*, log β_2 (in water) = 19.1 for dmp [14], 19.8 for dmdpp [15], and 16.5 for bq [14].   In addition, the extractability of Cu(I) was found to be dependent on the
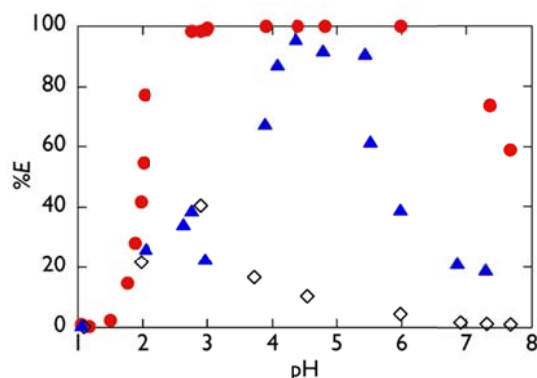

Figure 4.   Extraction of Cu(I) with 5.0×10^{-4} M bidentate amines in DCE in the presence of 5.0×10^{-4} M GSH and 0.10 M (H,Na)ClO$_4$.   [Cu] = 5.0×10^{-5} M, [buffer] = 0.01 M, shaking time = 30 min.   (●) dmp, (▲) dmdpp, (◊) bq.

shaking time in a certain pH range.   The %*E* values for every amine were somewhat increased by prolonging the shaking time by 60 min, *e.g.*, %*E* = 93 at pH 7.4 for dmp, %*E* = 35 at pH 7.4 for dmdpp, %*E* = 6 at pH 7.3 for bq.   This suggests that the ligand exchange between Cu(I)-GSH and L in the aqueous phase is relatively slow (cf. Figure 5).   Since the distribution constant of hydrophobic amines such as dmdpp and bq must be very high, although no data have been reported, the aqueous concentration
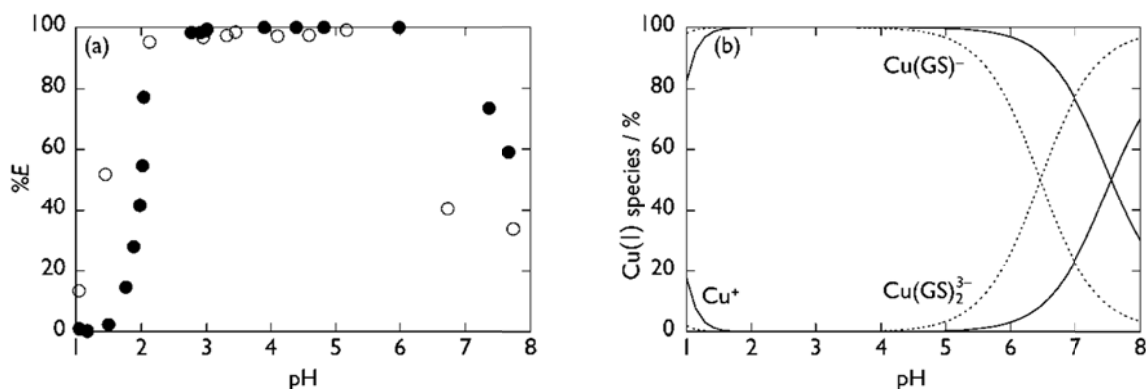
Figure 5. (a) Effect of the GSH concentration in the extraction of Cu(I) with 5.0×10^{-4} M dmp in DCE in the presence of 0.1 M ClO_4^-. [Cu] = 5.0×10^{-5} M, [GSH] = ($\bullet$) 5.0×10^{-4} M and ($\circ$) 5.0×10^{-3} M. (b) Fraction of Cu(I) species as a function of pH. [Cu]$_{ini}$ = 5.0×10^{-5} M, [GSH]$_{ini}$ = (solid lines) 5.0×10^{-4} M and (dotted lines) 5.0×10^{-3} M.

of dmdpp and bq is much lower than that of dmp. This probably affects the extractability of Cu(I) with different L at the shaking time of 30 min as shown in Figure 4. In fact, the %E value in the dmp system reached more than 98% within 5 min at around pH 4 and agreed with that expected from the back extraction because the percent back-extraction obtained was 1-2% at pH 3-6. Therefore, it is suggested that extraction equilibrium in the dmp system was reached under the above conditions.

The effects of the concentration of GSH in the aqueous phase and dmp in the organic phase on the extraction of Cu(I) were investigated. As shown in Figure 5(a), when the initial GSH concentration is high, a higher extractability at low pH but a lower extractability at high pH are obtained. Since the Cu(I) ions are present as GSH complexes in the aqueous phase as described above, GSH essentially competes with dmp and acts as a masking agent for Cu(I). The concentration of GSH complexing anions increases with an increase in pH because the pK_a of GSH is 2.43, 3.48, 8.62 and 9.43 [13]. On the other hand, quantitative extraction of Cu(I) was achieved from pH 2 to 8 when the dmp concentration was increased to 5.0×10^{-3} M (figure not shown), indicating the expected concentration dependency of dmp on the extraction efficiency of Cu(I). In Figure 5(b), the theoretical distribution of the Cu(I) species in the presence of GSH in the aqueous phase is given as a function of pH. The molar fraction values of the Cu(I) species were calculated using the pK_a of GSH and the stability constants (β_1 and β_2) for the Cu(I)-GSH complexes [13]. The extraction curves in Figure 5(a) correspond well to the distribution curves of the Cu(I) species in Figure 5(b). The main species, Cu(GS)$^-$, can readily react with dmp and be extracted as a dmp complex into the organic phase, while the Cu(GS)$_2^{3-}$ species seems to resist a formation of the dmp complex in the higher pH region.

The substoichiometric extraction of Cu(I) with dmp or ClO_4^- in the presence of GSH was used to determine the composition of the extracted Cu(I) complex. The extraction of Cu(I) with a substoichiometric concentration (1.0×10^{-4} M) of dmp in the presence of 1.0×10^{-1} M ClO_4^- and 5.0×10^{-4} M GSH in the aqueous phase at pH 4 was applied to a series of solutions containing different amounts of Cu(I). Figure 6(a) shows that the Cu(I) concentration in the organic phase increases with the increase in the amount of Cu(I) in the aqueous phase up to the equivalence point of [Cu(I)]/[dmp] = 0.5. Beyond this point, a constant amount of Cu(I) is extracted into the organic phase, *i.e.*, substoichiometric extraction

was achieved.   Its reproducibility was high at a relative standard deviation (RSD) value of 0.97 %.
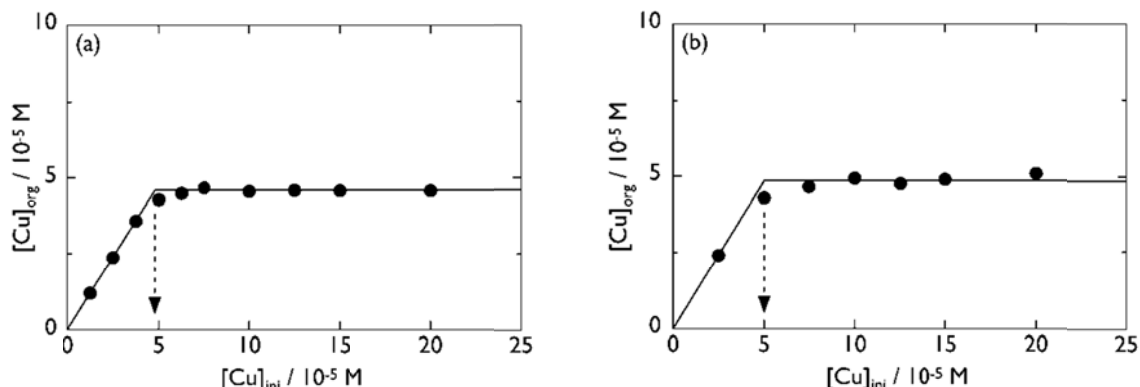

Figure 6.   (a) Substoichiometric extraction of Cu(I) with 1.0×10^{-4} M dmp in CHCl$_3$ in the presence of 1.0×10^{-1} M ClO$_4^-$ and 5.0×10^{-4} M GSH. pH 4.09 - 4.32.   (b) Substoichiometric extraction of Cu(I) with 5.0×10^{-5} M ClO$_4^-$ in the presence of 5.0×10^{-4} M dmp in CHCl$_3$ and 5.0×10^{-4} M GSH. pH = 4.14 - 4.33, ionic strength = 0.1 M ((H,Na)$_2$SO$_4$).

Consequently, as expected, the extracted Cu(I)-dmp complex was confirmed to be Cu(dmp)$_2^+$.   Also Figure 6(b) shows the substoichiometric extraction of Cu(I) using a combination of the substoichiometric concentration (5.0×10^{-5} M) of ClO$_4^-$ and an excess (5.0×10^{-4} M) of dmp in the presence of 5.0×10^{-4} M GSH at pH 4.   A constant amount of Cu(I) is extracted reproducibly (2.37 % as RSD) after the equivalence point of [Cu(I)]/[ClO$_4^-$] = 1.   These results provide evidence of the extraction of the ion-pair complex, Cu(dmp)$_2$ClO$_4$.

4. Conclusion

It was found that GSH is an effective reducing agent for the preparation of aqueous Cu(I) solutions. The time-course measurement of the absorbance change indicate that the reduced state of Cu(I) is stabilized by the complexation with GSH even under aerobic conditions for up to several hours.   The result obtained in this study demonstrates that quantitative extraction of Cu(I) from the aqueous solutions can be accomplished in the presence of GSH.   The present extraction method can be used for the substoichiometric extraction of Cu(I) with either dmp or ClO$_4^-$ and is expected to be applicable to the speciation of copper(I, II).

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