

Structural Study of Cu(II):Glycine Solution by X-ray Absorption Spectroscopy

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Abstract. This work investigated the Cu *K*-edged XANES spectra of copper(II) chloride aqueous solution mixed with glycine aqueous solution by varying Cu(II):Glycine molar ratio with the XANES spectra of standard reference of copper and starting solution of CuCl₂. Structural changes in these solutions are probed by X-ray absorption near-edge structure (XANES) spectra recorded in the vicinity of the copper *K*-edge for various Cu(II):Glycine molar ratios and for different pH. Comparison of spectra recorded for low and high concentrations of CuCl₂ in the mixed solutions show rather different XANES profiles. Low concentration of CuCl₂ in the mixed solutions without pH adjustment give also different XANES profiles from those obtained at a pH adjusted to 5.0. This highlights the importance of controlling these parameters for a proper determination of the various forms of metal complexes present in the mixed solutions.

1. Introduction

Metal ions play an important role in various functions of biological processes. In living organisms, most metal cations interact with proteins and can activate enzymatic properties. In human body, copper, which is the element investigated in our study, is the third most abundant transition-metal, following iron and zinc and mainly exists in the divalent oxidation number [1]. There was a previous report mentioning about the binding of copper with N-terminal region which is especially rich in histidine and glycine residues [2]. Glycine is the simplest form of amino acids, and our study focuses on its binding with Cu(II) using X-ray absorption near edge structure (XANES) technique. More generally, the knowledge of the metal-amino acid interaction is of crucial importance for a better understanding of various chemical processes occurring in living organisms. Metal toxicity or complexes transportation are typical effects that be influenced by some properties such as concentration or pH as we are probing here.

Amino acids consist of at least one amino group (NH₂) and a carboxylic acid group (COOH). They are the basic structural units of proteins and therefore constitute the building blocks of living cells. Glycine, H₂NCH₂COOH, the simplest amino acid and exists in different forms depending on the pH of the solution in which it is diluted: as glycinium cation for low pH (protonated form), as glycinate anion for high pH (deprotonated form), or as a zwitterion for 2.35 < pH < 9.78 (Figure 1).

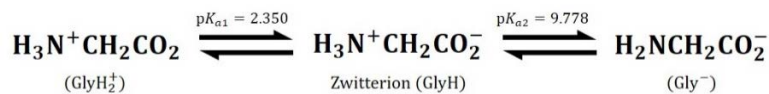


Figure 1. Chemical structure of glycine according to the pH of the solution [3].

Glycine contains two atoms being capable of creating chemical bond with a metallic center: the nitrogen of the amine group and one oxygen of the carboxylic acid group. In addition, one glycine can form two bonds with copper(II) cation and acts as a chelating ligand as shown in Figure 2.

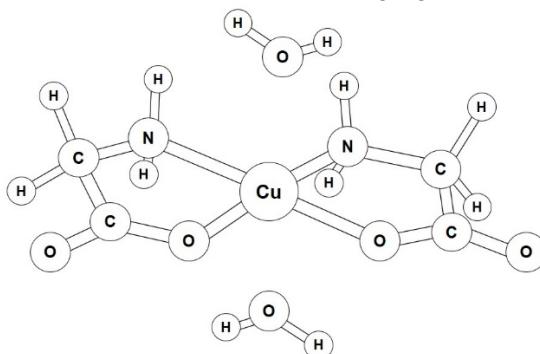


Figure 2. Illustration of copper – glycine complex [7].

Using the Window factor analysis (WFA) method, Darj and co-workers [3] have determined the different types of Cu(II)Gly complexes potentially present in solution for various pHs ranging from 1 to 7, and for a rather low copper concentration (i.e. 0.002M) and 0.5M glycine concentration. It is found that in addition of Cu^{2+} , CuGlyH^{2+} , CuGly^+ , and CuGly_2 can be formed in this large pH window. In the present study, two solutions are prepared at much higher copper concentrations of 0.25M and 0.5M, leading to an acidic medium of pH close to 2.0. Two other solutions are prepared with lower copper concentrations of 0.0015M. One of this solution has an unadjusted pH close to 3.0 and the other one has an adjusted pH close to 5.0.

2. Materials and Methods

Pure CuCl_2 salt and glycine powder were prepared separately by dissolution in pure deionized water. Mixed solutions of copper-glycine, (simply mentioned as Cu(II):Glycine throughout this study) were prepared with different ratios of 1:1 to 1:5 and for each solution the pH was measured. The samples were prepared in poly propylene plastic bags, further placed in superlene holder (200 μl) inserted in a stainless block.

The Cu *K*-edge absorption spectra (XAS) were measured at the beamline 1.1W of the Synchrotron Light Research Institute (SLRI), Nakhon Ratchasima, Thailand. The SLRI storage ring operated at an energy of 1.2 GeV, with the electron beam current between 140-80 mA. The beamline provides hard X-rays (4 to 18 keV) by means of a 2.2 Tesla Multipole Wiggler. The synchrotron radiation was monochromatized by a commercial double-crystal X-ray monochromator equipped with Si(111) crystals. The photon energy was calibrated using a copper foil as a reference. XANES spectra were measured in the transmission mode using ionization chambers as detector for high copper concentration of the mixed solution. Fluorescence mode using a Canberra 19-element Ge solid state detector was preferred for low concentration of the mixed solutions. The photon energy scan was carried out with the energy step width of 0.2 eV and with the time step of 1s. Sample integrity after about 0.5 h exposure to monochromatic synchrotron radiation was determined by monitoring the absorption spectra on sequential scans. The non-influence of the propylene plastic bags was checked on the same photon energy range.

The information of neighboring distance and coordination numbers surrounding the Cu atom was deduced from XANES spectra. Data reduction and analysis were carried out by using Athena software and Artemis software, respectively [4].

3. Results and discussion

Investigations of all aqueous solutions have been carried out using X-ray absorption spectroscopy. The obtained spectra are compared with the XAS spectra from Cu foil (0 oxidation state) from CuO (+2 oxidation state) and from CuCl₂ (+2 oxidation state) as shown in Figure 3. For clarity, the absorption spectra are normalized and offset vertically by 0.2. Interestingly, the absorption spectrum of the mixed solution is rather close in shape to the one of CuO. From the Cu(II):Glycine spectrum, we note 5 positions A to E, which can be assigned to transitions as discussed in reference [8]. In particular, A corresponds mostly to the transition Cu1s to 4p+ligand-metal charge-transfer (shake down transition type as described in [6]), and B - C mostly to the main transitions Cu1s to 4p, with the 4p being split in 2 groups.

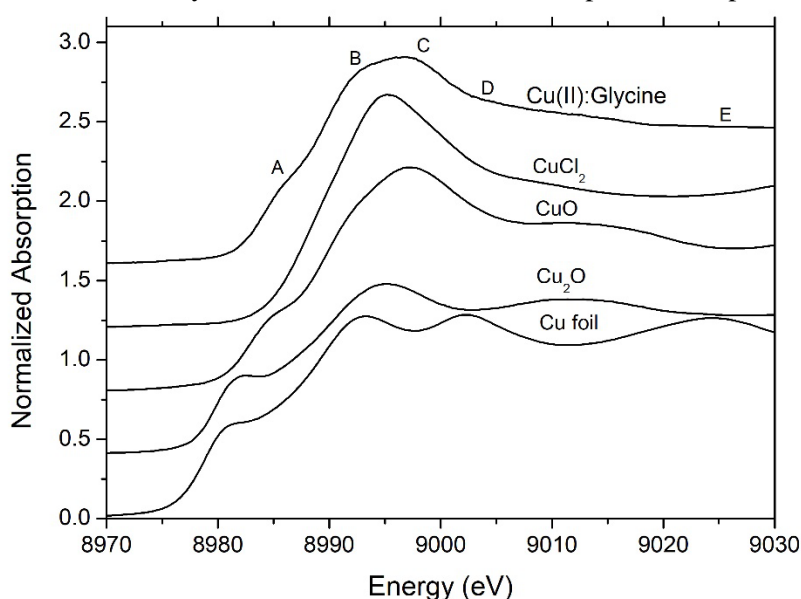


Figure 3. Cu K-edge XANES spectra of Cu foil, Cu₂O, and CuO standard sample and CuCl₂, and Cu(II):(Glycine sample solution).

Overview:

The strong similarities of the absorption spectra demonstrating in Figure 4a and 5a suggests that in this range of copper concentrations (i.e. 0.25M and 0.5M) the bonding efficiency between copper and glycine are comparable. A comparison of these two sets of spectra with the absorption spectra shown in Figure 6a which corresponds to low concentration values of copper and glycine, suggests that the bonding of the ligand with the metal ion is less efficient due to the very low intensity of the A structure and due to the disappearance of the B-C splitting of the main line. However, the adjustment of the pH at 5.0 has an interesting effect since – compared to Figure 6a, one clearly sees an increase of A intensity and a better splitting of B and C. This suggests that more metal-ligand complexes are formed in solution despite their low concentrations. One simple explanation is that at this pH value (pH=5>pK_{a1}), the glycine is mostly in its zwitterionic form with the carboxylic group being deprotonated and can thus easily form a bond with the metallic dication. In a more acidic medium and for pH< pK_{a1}=2.35, the situation is the opposite and the –COOH group cannot form a chemical bond with the metal. In Figure 4a and 5a, the increase of the A peak and the weak splitting of the B-C structures while the glycine concentration increases from 0 to 1.25M is attributed to an increase of the solution pH which should lead to slightly more zwitterionic form of glycine in solution, being able to fix the metallic ion with the deprotonated carboxylic group.

Fourier transform analysis:

Experimental XANES spectra of 0.25M copper chloride aqueous solutions, either pure or mixed with glycine aqueous solutions for concentrations ranging from 0.25M to 1.25M, are shown in Figure 4a. To collect information on the structure of copper in these solutions, Fourier transforms (FTs) analysis has been performed as shown in Figure 4b. The Fourier transforms have been calculated in the interval $k=3-9 \text{ \AA}^{-1}$. The first peak was observed at about 1.5 \AA and the second peak was found at about 2.3 \AA . Two peaks previously reported in the FT at 1.6 \AA and 2.4 \AA were identified as the Cu-glycine and Cu-O_{ax}, thus mentioning between that of copper and water oxygen atom at the axial position [6]. The intensity variation of second peak relied on the glycine molecule coordinated to copper and the positions of two water molecules [7]. The bond length observed at about 3.3 \AA coincided with the FT of the second hydration shell distance for the Cu-O_{solv} [7].

Two of five mixtures have pH values below 2.4, suggesting that the dominant copper species is CuGlyH²⁺. For the solutions Cu(II):Glycine from 0.25M:0.75M to 0.25M:1.25M, the pH values are between 2.40 to 2.51. With pH values higher than pK_{a1} of the carboxylic group of glycine, the zwitterionic form H₃N⁺CH₂CO₂⁻ defined as GlyH should be dominant and the concentration of the CuGly⁺ complex should increase.

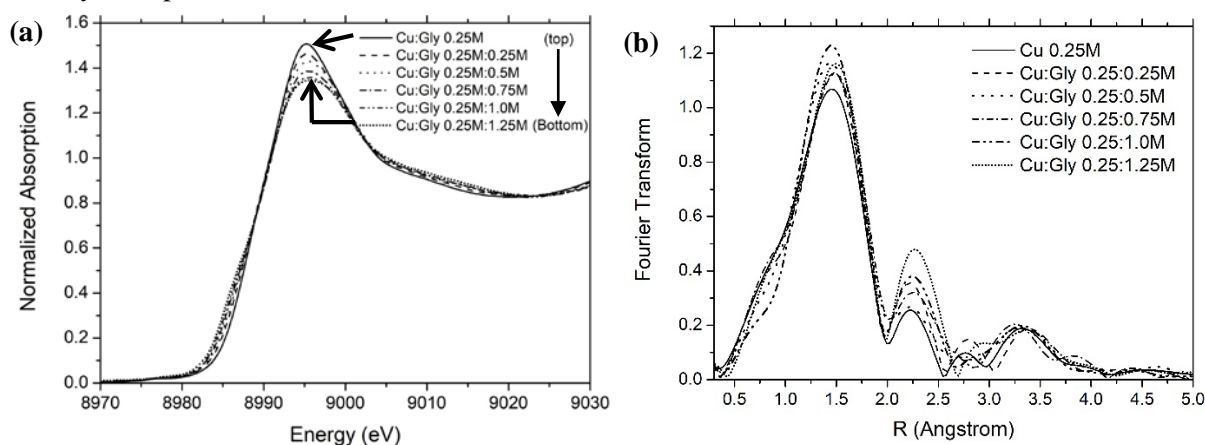


Figure 4 (a) Cu K-edge XANES spectra and (b) Fourier transforms at various molar ratios of 0.25mM Cu(II):Glycine.

A very similar trend is observed Figure 5a, corresponding to solutions having a concentration of copper of 0.5M, with glycine concentration ranging from 0.5M to 2.5M. For these solutions, the pH is measured between 1.90 to 2.36 (from low to high concentration of glycine). For pH values less than 2.4, the GlyH²⁺ species should be the dominant one leading to the main complex CuGlyH²⁺. Therefore, the first peak from EXAFS data analysis (Figure 5b) shows a gradual amplitude increase when increasing the concentration of glycine from 0.5M to 2.5 M.

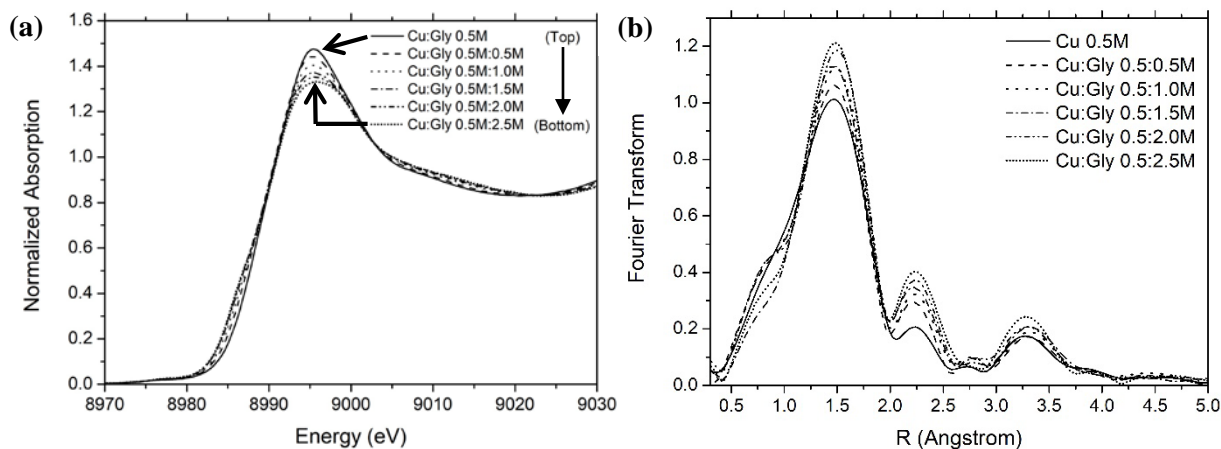


Figure 5 (a) Cu K-edge XANES spectra (b) Fourier transforms of the XANES spectra at various molar ratios of 0.5mM Cu(II):Glycine.

We show in Figure 6a the Cu K-edge absorption spectra for Cu(II):Glycine with solution 1.5mM:1.5mM, 1.5mM:4.5mM and 1.5mM:7.5mM concentration ratios. The corresponding pH values are 2.87, 2.90 and 2.98 respectively. Degree of water oxygen surrounded which associates with the first shell does not directly correlate with the increment of molar ratio of Cu(II):Glycine (Figure 6b), thus owing to the three copper species formed [3].

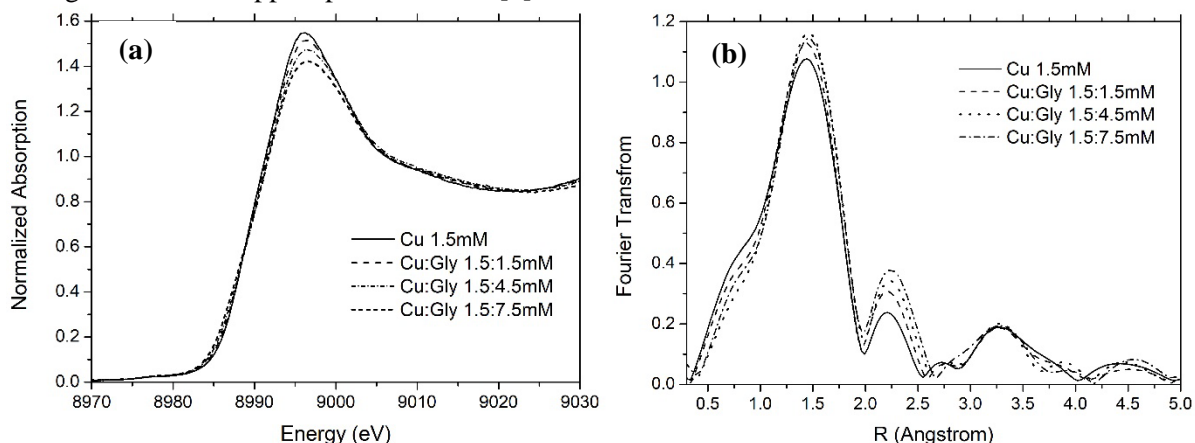


Figure 6 (a) Cu K-edge XANES spectra and (b) Fourier transforms of the XANES spectra at various molar ratios of 1.5mM Cu(II):Glycine.

Figure 7a shows the consistent of absorption feature changed when increase the molar ratio of Cu(II):Glycine, likewise observed in previous experiments. Moreover, this experiment clearly reveals the structural alteration occurred. When adjusting the pH value of 5.0 throughout the study, XANES spectrum becomes broader with the increment of molar ratio of Cu(II):Glycine (from 1.5mM:1.5mM to 1.5mM:7.5mM). This may due to the occurrence of the split main absorption line of copper complexed with glycine, referred to B and C as previously reported [8]. Moreover, these structural features are found in all previous results shown but the resemblances of these peaks, B and C are clearly observed with the adjustment of pH 5.0. Because of the influence of pH, the major species formed is CuGly_2 [3], thus significantly promoting this experimental spectrum. Besides peaks B and C found, the shoulder like feature (peak D) and the deep minimum (peak E) are apparently observed as well. As seen from Figure 7b, the degree of water oxygen surrounded (the peak around 1.5 Å) proportionally correlates with the increment of molar ratio of Cu(II):Glycine. In this experiment, at the molar ratio of

Cu(II):Glycine of 1.5mM:4.5mM, the peak around 3.9 Å is evidently appeared, thus assuming the Cu-O bond distance [1].

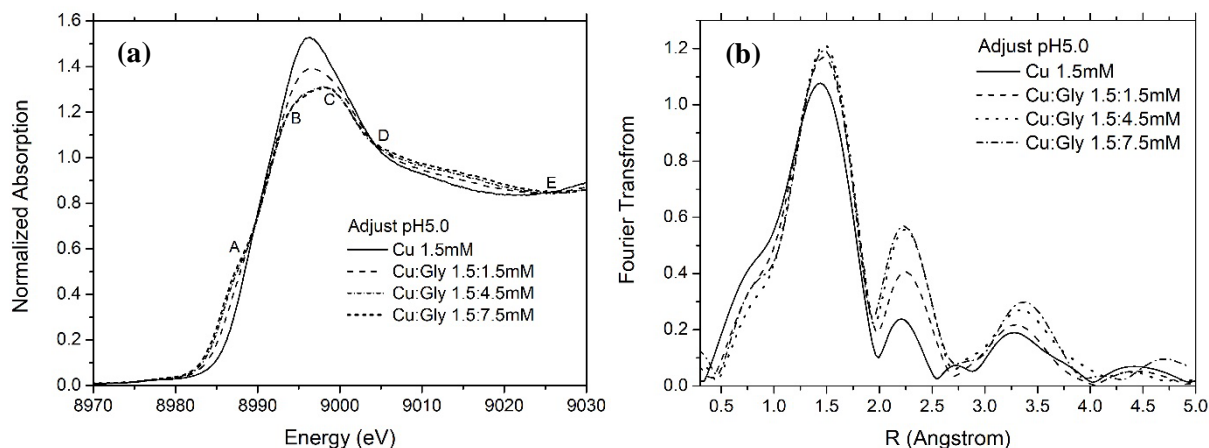


Figure 7 (a) Cu K-edge XANES spectra and (b) Fourier transforms at various molar ratios of 1.5mM Cu(II):Glycine when adjusted the pH value of 5.0.

4. Conclusions

We have reported the Cu K-edge XANES spectra of copper(II) chloride aqueous solution mixed with glycine aqueous solution by varying Cu(II):Glycine molar ratio. Investigation on XANES spectra of standard reference of copper and starting solution of CuCl₂ could indicated the presence of Cu(II):Glycine in aqueous solution. Moreover, characterization of the absorption spectra proposed the spectral fingerprint for Cu(II):Glycine complex as formerly studied [8]. Analysis of EXAFS data provided the structural information at different Cu(II):Glycine ratios. In particular, the pH values measured are important, thus causing major and minor copper species. Changes in pH value affected the coordination shell of the metallic center as observed from EXAFS results. Thereafter, the different molar ratio of Cu(II):Glycine were prepared and driven to the single major species of copper complex solution by controlling pH adjustment of 5.0. The results support that the species of copper complexed with glycine was pH dependent and affected the structural features investigated by both XANES and EXAFS. The benefit of this study is to understand the condition to form copper-glycine species. This would be a detailed information which supports the usage to employ this knowledge for further applications.

5. References

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