

Preparation of Copper (II)-Glycine Complex

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(Figs. 1-4)

As part of the study on the improvement of a synthetic milk, we have previously reported the methods for preparing iron- and zinc-complexes.¹⁾⁻³⁾ This paper intends to be one of this series and deals with the preparation of a copper(Cu)-glycine(Gly) complex. Several factors that influence this complex-formation are also examined.

Many workers have studied Cu(II)-Gly complexes. BORSOOCK and THIMANN⁴⁾ examined formation of the complexes by means of spectrophotometric and electrophoretic analyses, and found that a Cu(II)-Gly₂ complex was formed in alkaline solution. GOULD and VOSBURGH⁵⁾ investigated the composition of Cu(II)-Gly complexes formed in the reaction systems with different Cu(II)/Gly molar ratios; a [Cu(II)-Gly]⁺ or a Cu(II)-Gly₂ complex was formed in the system with the Cu(II)/Gly molar ratio of 1 : 1 or less than 1:2, respectively. KEEFER⁶⁾ determined dissociation constants of Cu(II)-Gly complexes by a potentiometric method and suggested that the complexes existed in the form of [Cu(II)-Gly₃]⁻ in the presence of excess Gly⁻ of 0.2 to 1.0 M in the system. As the forms of Cu(II)-Gly complexes in solution, QUINTIN⁷⁾ also reported as follows. No complexes were formed in strongly acidic solution below pH 2; however, in weakly acidic (pH 3~7) or in alkaline solution, both 1:1 and 1:2 or only 1:2 complex was formed, respectively. On the basis of the data for the proton displacement in the Cu(II)-Gly systems, MURPHY and MURTELL⁸⁾ assumed the structure of the complexes as follows:
$$\begin{array}{c} \text{H}_2\text{O} > \text{Cu(II)} < \text{NH}_2 - \text{CH}_2 \\ \text{H}_2\text{O} > \text{Cu(II)} < \text{O} - \text{C} = \text{O} \end{array}$$
 for the Cu(II)-Gly (1:1) complex and $\frac{\text{H}_2\text{C} - \text{H}_2\text{N}}{\text{O} = \text{C} - \text{O}^-} \text{Cu(II)}/2$ for the Cu(II)-Gly (1:2) complex. Stability constants and structures of the complexes were examined also by DOBBIE *et al.*⁹⁾ On the other hand, TOMITA¹⁰⁾ indicated a model for the crystal structure, which is based on the IR-spectral data.

From the biochemical view point also, a number of reports on Cu have been published, most of them are reviewed in "The Biochemistry of Copper".¹¹⁾ In the field of food chemistry, the phenomena based on the redox reaction of Cu are the major problems; e.g., the promoting effect of Cu on rancidity of edible oils¹²⁾ and on discoloration of anthocyan pigments,¹³⁾ or the antioxidative effects of Cu-protein and ascorbic acid¹⁴⁾ on these undesirable reactions.

MATERIALS AND METHODS

1. Reagents

Reagents used in this experiment were of a guaranteed grade, except for ethyl alcohol. The ethyl alcohol was 78~79°C fraction distilled from an analytical grade reagent.

2. Reaction systems

10^{-2} M CuSO_4 and 2×10^{-2} M Gly were used as stock solutions. Various reaction systems with different Cu/Gly molar ratios were prepared by mixing the stock solutions at a given ratio. The pH of these solutions was adjusted to a given value with 0.1N HCl or 0.1N NaOH.

3. Absorption spectra

Absorption spectra of each reaction solution were obtained with a Hitachi spectrophotometer Type 124.

4. Determination of Cu(II) and Gly

Cu(II) was determined with a Hitachi atomic absorption spectrophotometer Type 207.

Gly was determined by the Kjeldahl method.

5. Paper electrophoresis of Cu(II)-Gly complex

Paper electrophoresis was carried out with a Toyo electrophoresis apparatus No. C under the following conditions:

paper strips: Toyo Roshi No. 50 (2 x 40 cm)

buffer solution: $\text{Na}_2\text{B}_4\text{O}_7\text{-H}_3\text{BO}_3$ buffer (pH 8.40, $\mu=0.1$)

voltage: 500V, running time: 2hr

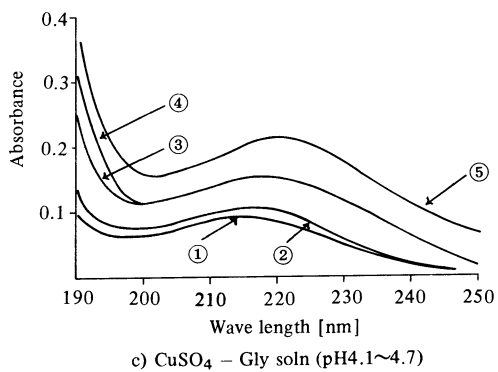
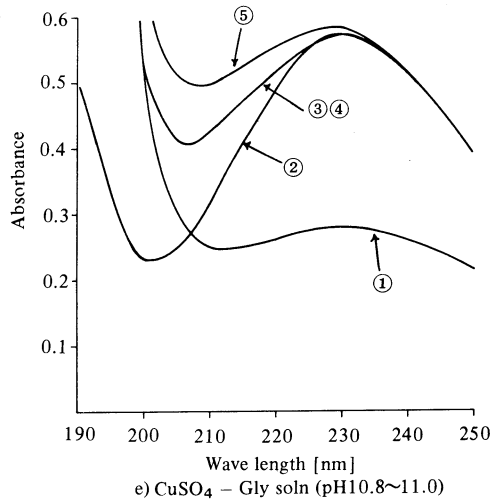
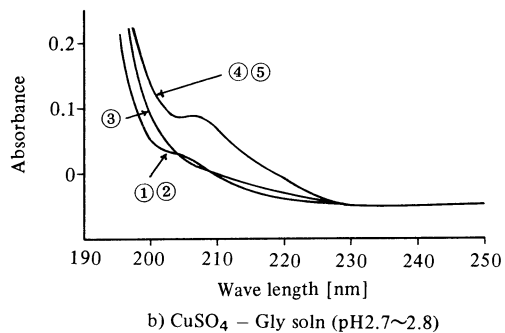
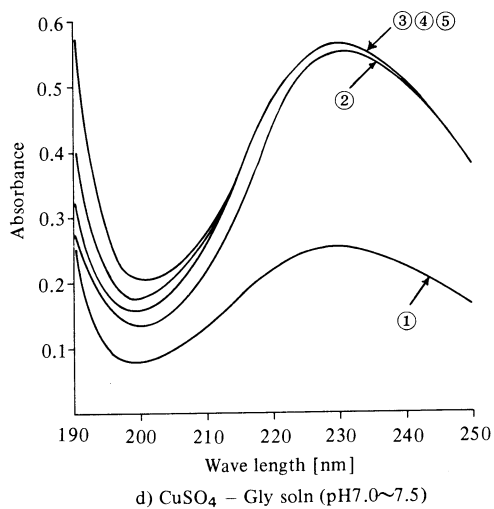
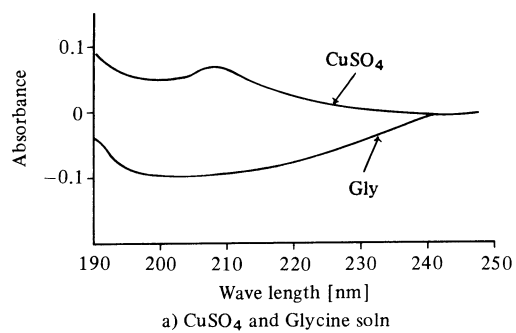
color reaction for Cu(II): 5% $\text{K}_2\text{Fe}_2(\text{CN})_6$ solution

color reaction for Gly: 0.5% ninhydrin/n-butanol solution

RESULTS

1. Formation of Cu(II)-Gly complex

The relation between the Cu(II)/Gly molar ratios and the pH values of the reaction systems was examined in order to detect the adequate conditions for preparing a Cu(II)-Gly complex. The reaction systems were of 20 types; five solutions with different Cu(II)/Gly ratios (Cu(II)/Gly=1:1, 1:3, 1:5, 1:7, and 1:10) were prepared and each of them was adjusted to pH 2.7, 4.5, 7.2, and 11 separately. The absorption spectra of these solutions were determined after standing for 30 min at $18 \pm 0.5^\circ\text{C}$ (Fig.1-b~e). Fig.1-a shows the spectrum of the control solution of CuSO_4 or Gly alone. On the CuSO_4 solution, an absorption maximum appeared at 210 nm. The spectra of the most acidic solution (pH 2.7) were similar to that of the CuSO_4 alone (Fig. 1-b). When the pH of the solutions was made slightly acidic (about pH4.5), the maximum peak shifted to longer wave-length; a small peak was found at 230 nm (Fig.1-c). This peak became higher with the increase in pH of the solution. This fact suggests the formation of a Cu(II)-Gly complex in the



- ① Cu/Gly=1/1
- ② Cu/Gly=1/3
- ③ Cu/Gly=1/5
- ④ Cu/Gly=1/7
- ⑤ Cu/Gly=1/10

Fig. 1 Absorption curves of various Cu-Gly solutions

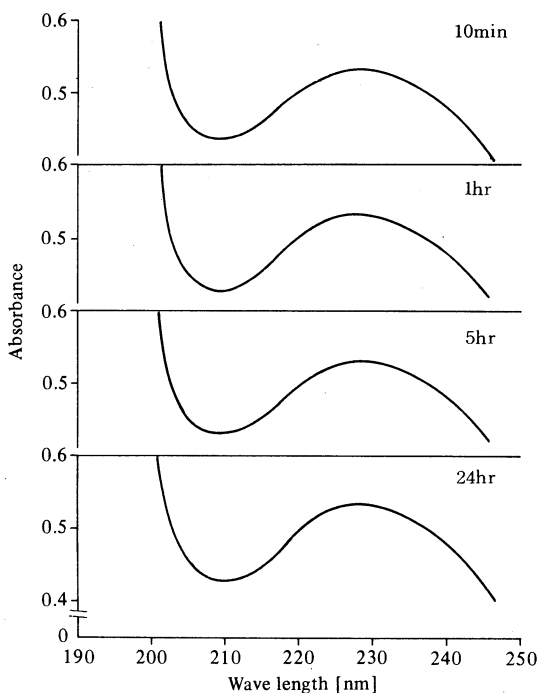


Fig. 2 Absorption curve of Cu(II)-Gly systems (Cu(II)/Gly=1/3, pH after preparation 11.0) over a period of 24hr.

reaction system. The values of λ_{\max} of these solutions were almost the same, except for that of the Cu(II)/Gly(1:1) system, the value of which was one half of those of the other systems. These results are of interest in connection with the structure of the Cu(II)-Gly complex in solution. The effect of the elapse of time was examined with the Cu(II)/Gly (1:3) system for 10 min~24 hr after preparing the system. No change was observed in the absorption curve. This result shows that the formation of Cu(II)-Gly complexes goes to completion within 10 min.

2. Preparation of Cu(II)-Gly complexes

On the basis of the above results, the method for preparing the Cu(II)-Gly (1:2) complex was designed as shown in Fig. 3. The complex obtained by this method was identified by paper electrophoresis as the expected one (Fig. 4).

3. Characterization of the Cu(II)-Gly complex

The contents of Cu and Gly in this preparation were 22.9g and 53.5g per 100g sample, respectively. When these values were converted to a molar ratio, the Cu(II)/Gly ratio became 1:2. From this, it is clear that the complex obtained is a Cu(II)-Gly (1:2) complex.

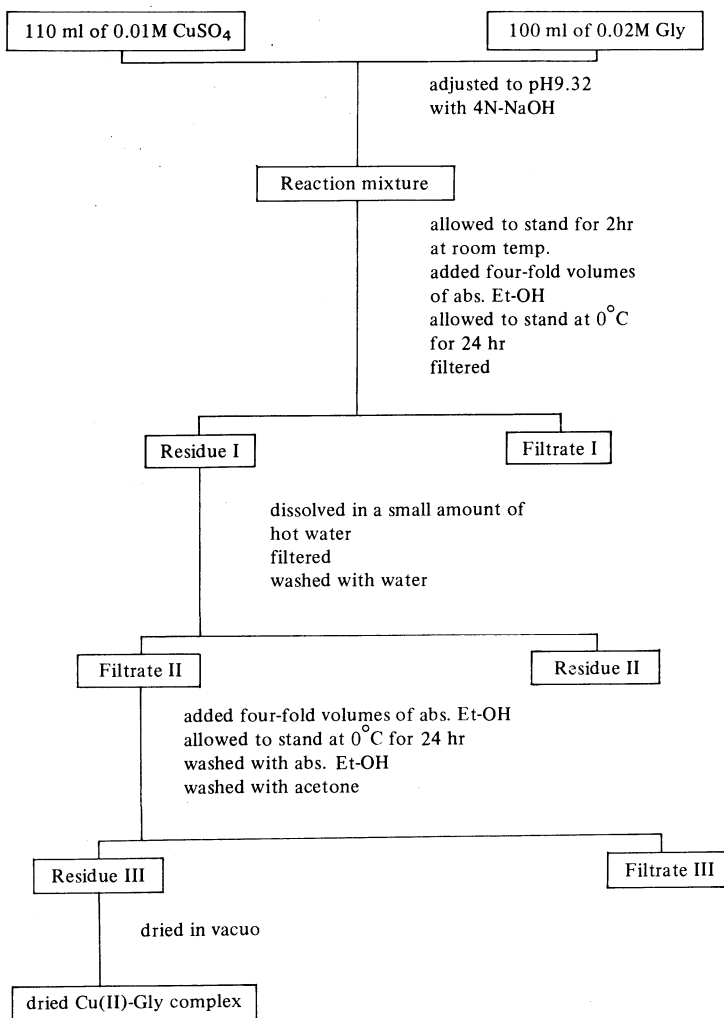


Fig. 3 Preparation of the Cu(II)-Gly complex

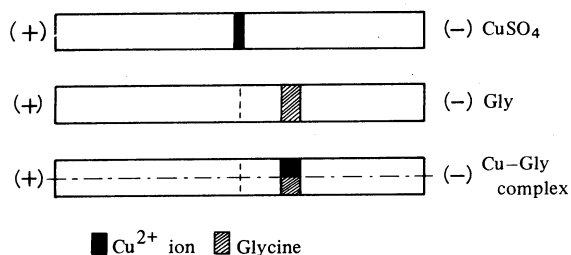


Fig. 4 Paper electrophoretograms of the Cu(II)-Gly complex

DISCUSSION

Addition of Gly to the control solution of CuSO_4 alone resulted in a shift in the absorption maximum from 210 nm to 230 nm. Such shifts apparently indicate the formation of a Cu(II)-Gly complex in the system. From the fact that the absorbance at 230 nm became more intense with the increase of pH in the solution, the ionic state of Gly in alkaline solution is considered to be a very convenient one for the complex-formation, because Gly ionizes as $\text{H}_2\text{C}-\text{COOH}$ / NH_3^+ in an acidic solution but as $\text{H}_2\text{C}-\text{COO}^-$ / NH_2 in an alkaline solution. Under the alkaline conditions, as the nitrogen atom of Gly has a superior tendency to the oxygen atom for the coordination to Cu, the formation of the Cu(II)-Gly complex must occur readily; the structure of the complex shown as $\text{H}_2\text{C}-\text{H}_2\text{N}-\text{Cu(II)}/2$. Although the absorption peak at 230 nm was small in weakly acidic solution (pH 4.5), it became larger by increasing the concentration of Gly in the reaction system. This fact suggests that the complex can be formed, even in a small amount, under acidic conditions below the iso-electric point of Gly.

According to DOBBIE *et al.*⁹⁾, the Cu(II)-Gly complex formed in neutral or alkaline solution is composed of one mole of Cu(II) and two moles of Gly. The result in this experiment is in good accord with their observation. In the Cu(II)/Gly (1:1) system, the amount of the complex formed was about one-half of those formed in the other systems. The reason for this is that the amount of Gly added was too small compared with that of Cu(II) in the system. On the basis of those observations, the method for preparing the Cu(II)-Gly (1:2) complex was designed as shown in Fig. 3, that is, 10^{-2} mole of CuSO_4 and 2×10^{-2} moles of Gly were mixed to give a Cu(II)/Gly molar ratio of 1:2. The preparation obtained was examined by paper electrophoresis and identified as the expected Cu(II)-Gly (1:2) complex.

YOSHINO *et al.*¹⁵⁾ examined the effect of pH on the form of Cu(II)-Gly complexes during the electrophoresis; the formation of a Cu(II)-Gly_2 or a $[\text{Cu(II)-Gly(OH)}_2]^-$ complex was proved in the pH range 9.34 to 10.40 or at pH 11.52, respectively. In the present experiment, the electrophoresis was carried out at pH 8.1. As can be seen Fig. 4, under these conditions, the Cu(II)-Gly (1:2) complex migrated to the cathode. As the Cu(II)-Gly (1:2) complex does not seem to have any positive charge at pH 8.1, the result is conflicting. On this point, further experiments using a high-voltage paper electrophoretic apparatus are being scheduled.

SUMMARY

A method for preparing a Cu(II)-Gly complex was presented on the basis of the examination of the complex formation. The preparation obtained was identified by paper electrophoresis as the expected Cu(II)-Gly complex being composed of Cu(II) and Gly in the molar ratio of 1:2.

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銅(II) — グリシン錯体の調製

今村 経明 ・ 畑中 千歳 ・ 堂々 隆史

人工乳のミネラルに関する基礎的研究に必要な研究材料となる種々の金属—アミノ酸錯体を調製してきたが、今回は、銅(II)—グリシン錯体の調製を試みたのでその結果を報告した。

銅(II)—グリシン錯体についてはこれまでに、さまざまな視点から多くの研究が行なわれている。本報では、銅(II)—グリシン錯体標品を得るためにまず銅(II)—グリシン錯体の生成条件について検討し、中性からアルカリ性域にかけて生成する銅(II)—グリシン錯体は $[\text{Cu(II)-Gly}_2]$ の形で存在することを分光光学的に確かめた。その他、温度、反応時間、銅(II)/グリシン比などについても検討し、それらの結果をもとに、錯体の調製法を決めた。そしてこの方法で得られた標品は、銅(II)：グリシン = 1：2の組成比をもつ銅(II)—グリシン錯体であることを確認した。