

Formation, Chemical Structure, and Preparation of Zinc-Cysteine Complex

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(Fig. 1-5)

We previously showed that the content of zinc (Zn) in human milk is significantly higher than in cow's milk powder that is commercially supplied for nourishing infants.¹⁾ Recently, deficiency of Zn has been reported to may cause the infants to acrodermatitis enteropathica.²⁾ Hurley et al.³⁾ reported that human milk contains a Zn-binding substance which promotes absorption of Zn in the intestine. Some investigators consider that this substance is a peptide,⁴⁾ whereas others claim that this is citric acid⁵⁾ or picolinic acid.⁶⁾ However, which substance is the conclusive factor has not been decided. We have noticed to cysteine (CySH) as a novel ligand because it is contained in human milk at higher level than in cow's milk due to the difference of protein composition. On the other hand, we have studied on the distribution of Zn in soybean grains and found that CySH is an effective Zn-ligand to forms a low molecular Zn-compound.⁷⁾ Therefore, knowledges on the properties of Zn-CySH complex is important in understanding how and to what degree CySH may take part in carrying Zn. The precise structure and chemical properties of the Zn-CySH complex have so far remained unclear. Here, we describe the conditions of forming Zn-CySH complex and propose the chemical structure of the complex.

Material and Methods

1. Reagents. All reagents used were of guaranteed grade.
2. Reaction systems. Stock solutions of ZnCl_2 and CySH were prepared at concentrations of 5×10^{-2} and 2×10^{-3} , respectively. By mixing them in suitable proportions, a series of ZnCl_2 -CySH solutions with different molar ratios of Zn and CySH were prepared. The pH of each solution was adjusted to a desired value by adding 0.1N NaOH or HCl, and the ionic strength was adjusted to 0.1 with NaClO_4 . Nitrates have been generally used for examining metal-complex formation, but the chloride form was used in this work because dietary Zn should be converted to ZnCl_2 in the stomach.
3. Examination of Zn-CySH complex formation. Two different methods were employed for determining the metal complex formation. The one was potentiometric titration method and it was carried out with a Hiranuma RAT-101S apparatus. The

titration was performed with 5×10^{-3} N NaOH under a stream of N_2 gas. The other method was electrophoresis and it was carried out at 30 V/cm for 20 min. at room temperature using a Toyo TPE-406 high-voltage paper-electrophoresis apparatus. Sheets of Toyo filter paper No.51A (40 cm length and 2 cm width) were used. After the electrophoretic migration, the sheets were dried up and divided into two strips by cutting them lengthwise. One of the two strips was sprayed with a ninhydrin-containing solution to detect CySH, and the other with a chloroform solution containing dithizone to detect Zn.

4. Determination of CySH. The amount of CySH was estimated from the value of nitrogen obtained by a micro Kjeldahl method.

5. Detection of free SH-group. Nitroprusside reaction was applied to detect free SH-group, i.e., 5 ml of 1% sodium nitroprusside was added to 10 ml of a sample. Intensity of the developed color was determined at 515 nm with a Hitachi 124 absorption spectrophotometer.

Results and Discussion

1. Formation of Zn-CySH complex. Potentiometric titration curves of four reaction systems which contained Zn and CySH in the molar ratio of 1:1 (1/1), 1:2 (1/2), 1:3 (1/3), and 1:4 (1/4), together with the control system (1/0) which contained $ZnCl_2$ alone, are shown in Fig. 1. In the control system, a white gel-like precipitate was formed during the course of titration process. Such a precipitate is commonly observed when a metal salt solution is made alkaline, and this is produced by hydrolytic polymerization of the metal aquo ion. The polymerization may be prevented by complex formation of the metal ion with a ligand which has higher coordination ability than H_2O . Therefore, the fact that any precipitate was not produced in system 1/2, 1/3, and 1/4 indicates that Zn-CySH complex was formed. The precipitate was also observed in system 1/1. This is due to the insufficiency of the amount of CySH as described later.

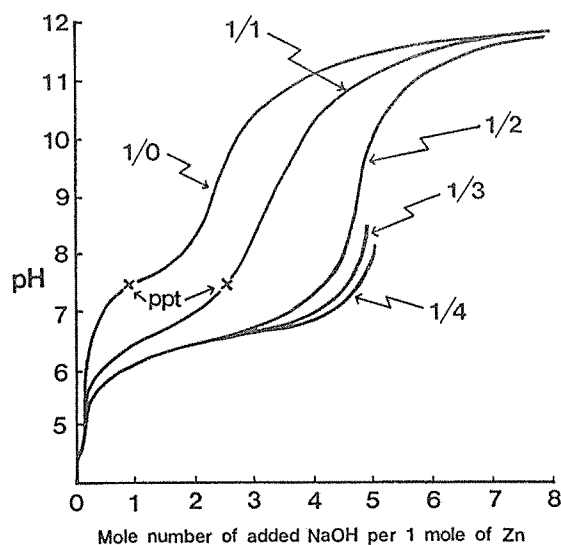


Fig. 1 Potentiometric titration curves of $ZnCl_2$ -CySH solutions with different ratio of Zn/CySH. Fractions in parentheses represent Zn/CySH ratio of each solution.

The pH of reaction system 1/1–1/4 did not rise so higher than that of control system 1/0, as shown in Fig. 1. This supports the possibility of Zn–CySH complex formation because, when a metallic element forms complex with a ligand, H atoms of the ligand are replaced by the metal ion and the released protons lower the pH value of the solution.

The more direct evidence of the complex formation was obtained from the results of a high-voltage paper-electrophoresis (buffer: 0.05M sodium borate containing 0.13M KCl, pH 10.5), as shown in Fig. 2. There was a band in the system 1/2 at which both Zn and CySH were detected, and the band was different from that of either Zn or CySH alone. The fact suggests that Zn and CySH migrated together as a complex. Additional bands to the above one were also observed in the electrophoregrams of system 1/1, 1/3, and 1/4. Their bands are due to excess Zn or CySH, which will be discussed in the next section.

In order to examine the effect of pH on the Zn–CySH complex formation, electrophoresis of the system 1/2 was performed by varying the pH values from 3.7 to 10.5. The electrophoretic patterns are shown in Fig. 3. The same band as that found in Fig. 2 which contains both Zn and CySH, was observed in the samples of pH 9.5 and 10.5. The complex formed under the alkaline conditions was anionic because the complex migrated toward the anode. Such evidence for the Zn–CySH complex formation was not detected in the sample of pH 3.7. In the sample of pH 6.2 and 7.5 which are physiologically important conditions, both Zn and CySH did not migrate and detected at original point. The fact suggests the existence of electrically neutral Zn salt – CySH mixture or Zn–CySH complex.

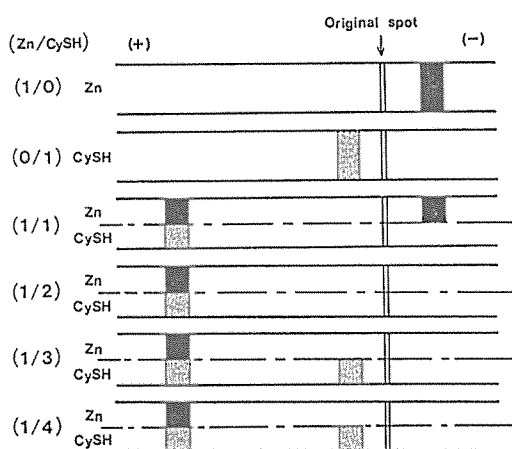


Fig. 2 Electrophoretic patterns of $ZnCl_2$, CySH, and four types of Zn–CySH solutions with different Zn/CySH ratio. Buffer solution: 0.05M Na-borate contg. 0.13M KCl (pH 10.5)

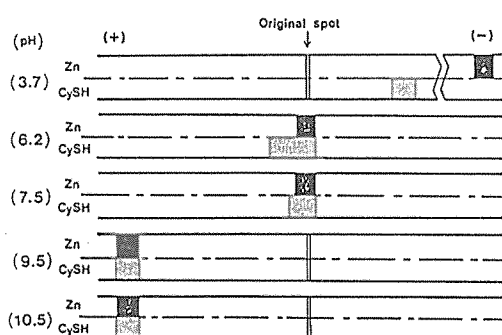
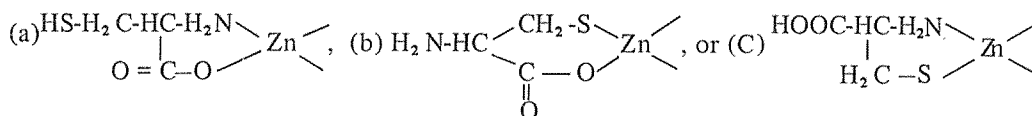


Fig. 3 Effect of pH on electrophoretic patterns of $ZnCl_2$ -CySH (Zn/CySH=1/2) solutions. Buffer solutions: n-butyl alcohol-acetic-NaHCO₃ (pH 3.7) n-butyl alcohol- NaCO₃-NaCl (pH 6.2) pyridine- sodium tartarate (pH 7.5) sodium borate- KCl (pH 9.5) sodium borate- KCl (pH 10.5)

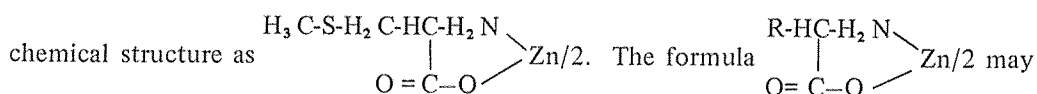
2. Composition of the Zn-CySH complex. As shown in Fig. 1, the amount of the added NaOH up to the inflection point increased with the increment of CySH and this amount in the system 1/2 was twice that in the system 1/1. The amount of NaOH required to reach the inflection points the systems 1/3 and 1/4 were roughly equal to that in the system 1/2. Accordingly, the appropriate ratio of Zn/CySH for the complexation is expected to be 1/2. The content of Zn in the system 1/1 was in excess and that in the system 1/3 or 1/4 was insufficient in amount to form the complex with CySH. The precipitate formed at 2.5 mole NaOH in the system 1/1 is due to excess $ZnCl_2$ (Fig. 1) and the Zn-containing band in electrophoregram other than that of Zn-CySH complex (Fig. 2) is due to free Zn. On the contrary, the amount of CySH in the system 1/3 and 1/4 might exceed the appropriate level, and one of the two CySH-containing bands in Fig. 2 is due to the excess CySH. The facts suggest that the Zn-CySH complex is composed of 1 mole of Zn and 2 mole of CySH. This is also supported by the results of the nitroprusside test which will be described later.

3. Chemical structure of the Zn-CySH (1:2) complex. Zn has four coordination number and CySH is tridentate ligand containing $-COOH$, $-NH_2$, and $-SH$. In the case of Zn-CySH (1:2) complex formed under alkaline conditions, the possible chemical structures are illustrated as



The thiol group of CySH is in the free state in the formula(a), while it binds to the Zn atom in the formulas(b) and (c). The nitroprusside test which detects free SH groups was carried out to discriminate the true structure and the results are shown in Fig. 4. Color was not developed in the systems 1/1 and 1/2, that is, free thiol groups did not exist in these systems. The systems 1/3 and 1/4 were positive on the color reaction, and their intensities differed according to the excess amount of CySH to the formation of Zn-CySH (1:2) complex. These data suggest that the thiol group was involved in the complex formation and therefore the formula(a) was excluded. The structure, in addition, is anionic since the complex migrated toward the anode in the electrophoresis under alkaline condition. Formula(c) satisfies the above requisites. The involvement of SH group in the complex formation under alkaline conditions is reasonable on the viewpoint of the dissociation constants of CySH, i.e., pK_1 1.96 (COOH), pK_2 8.48 (NH_2), and pK_3 10.55 (SH).⁸⁾

An electrically neutral complex has been reported by Shindo & Brown.⁹⁾ They prepared Zn-(S-methyl-CySH) complex in an acidic solution (pH 1-2) and proposed its



be applicable to every Zn complex with α -amino carboxylic acids of which amino and carboxyl groups are in free state. A compound like the neutral complex was observed in the sample of pH 6.2 and 7.5. On the basis of the dissociation constant of CySH as

described above, a reasonable chemical structure of the complex is

$$\begin{array}{c} \text{HS-H}_2\text{C-HC-H}_2\text{N} \\ | \\ \text{O}=\text{C}-\text{O} \end{array} \text{Zn}/2.$$

However, identification of the neutral complex was not tried. The possibility is also considered that any complex was not formed and undissociated ZnCl_2 and CySH existed on the state of mixture. In the present investigation, any complex was not formed under strongly acidic condition.

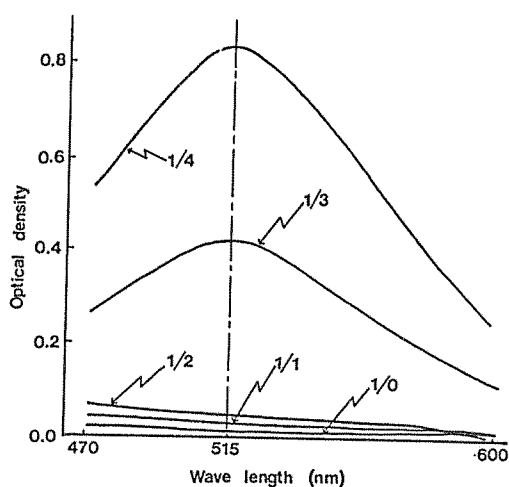


Fig. 4 Comparison of the color intensity by nitroprusside reaction among five types of ZnCl_2 - CySH solutions with different Zn/CySH ratio. Fraction in parentheses represent the Zn/CySH ratio.

4. Preparing the Zn-CySH (1:2) complex and its identification. In order to prepare the Zn-CySH complex most efficiently, the time required for the formation of the complex was examined by comparison of the potentiometric titration curves of the following samples. All of the samples used were made up of Zn and CySH in the ratio of 1/2, the pH was adjusted to 9, and kept for various periods. The results are shown in Fig. 5. The titration procedure was performed within four min. The curves of all the samples were nearly the same and irrespective of the reaction periods. Accordingly, the complex formation is considered to be completed within a short time and the complex remained stable for at least several hours.

An example of preparing the Zn-CySH complex is as follows. 50 ml of 0.5M ZnCl_2 and 100 ml of 0.5M CySH were mixed and pH of the mixture was adjusted to 9 with 1 N NaOH . The solution was allowed to stand for 30 min. at room temperature. Then, 600 ml of 99% ethanol was added and the solution was kept for 24 hr at 5°C . The cold solution was centrifuged for 20 min. at 3,000 rpm and the precipitate was collected.

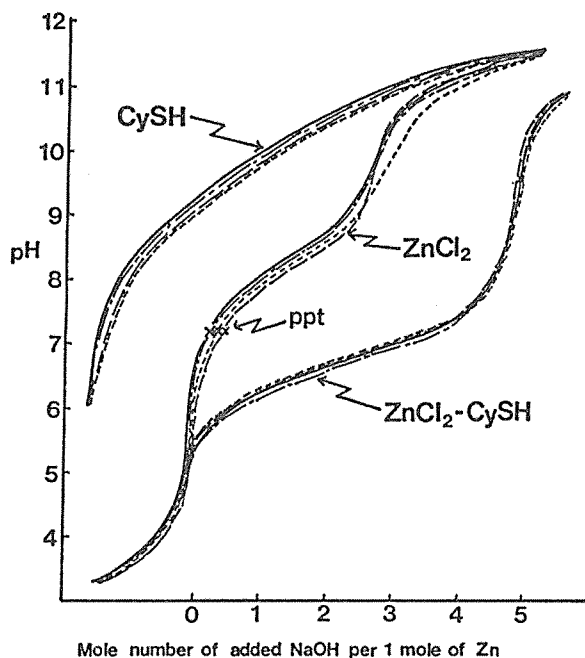


Fig. 5 Potentiometric titration curves of $ZnCl_2$ - $CySH$ ($Zn/CySH=1/2$) solutions differed on the elapsed time after mixing.

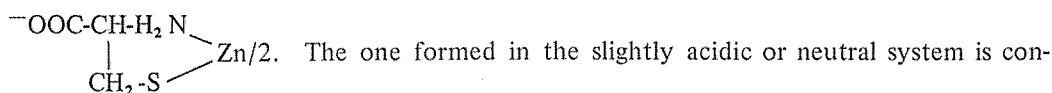
— immediately after mixing
 - - - 30 min. after mixing
 - · - 60 min. after mixing
 · · · 120 min. after mixing

The precipitate was dissolved in water again and the above precipitation procedure were repeated several time for purifying the preparation. Final precipitate was dried in vacuo and a white powder was obtained. The white powder was identified as Zn - $CySH$ complex by means of the high-voltage paper-electrophoresis, and it was composed of Zn and $CySH$ in the ratio of 1:2. The yield was 83% of theoretical value.

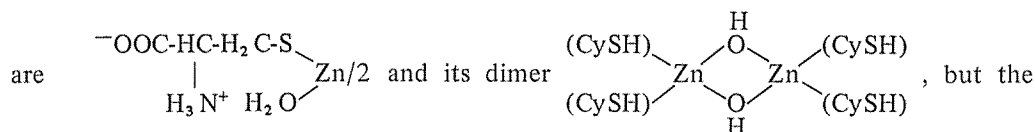
Summary

As one of our continuous investigations on the interaction of minerals with food components, the present work deals with the complex formation between zinc (Zn) and cysteine ($CySH$). The composition and chemical structure of the complex between Zn and $CySH$ have been investigated, and Zn - $CySH$ (1:2) complex has been elucidated by analyzing various types of $ZnCl_2$ - $CySH$ solution as reaction systems.

The Zn - $CySH$ complex formed in alkaline reaction systems was composed of Zn and $CySH$ in the ratio of 1:2, and its chemical structure is considered to be



sidered to be

$$\begin{array}{c} HS-H_2C-CH_2-H_2N \\ | \\ O=C-O \end{array} \left. \begin{array}{l} \\ \\ \end{array} \right\} Zn/2. \text{ Other chemical structures of the neutral complex}$$


reliability is scarcely. In the strongly acidic system, any complex was not detected. On the basis of the results, a method for preparing Zn-CySH (1:2) complex under alkaline condition was examined.

References

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亜鉛－システイン錯体の形成，化学構造ならびに調製

佐藤 明・谷本幸照・今村経明

食品成分とミネラルの相互作用に関する研究の一部として，今回は亜鉛（Zn）とシステイン（CySH）の錯形成を取り上げ，その組成および化学構造も検討した。そして混合比が異なる各種の $ZnCl_2 - CySH$ 溶液を用い，まず組成比が $Zn : CySH = 1 : 2$ であることを明らかにした。次に配位基について検討し

アルカリ性反応系で形成される $Zn - CySH (1 : 2)$ 錯体の化学構造は

$$\begin{array}{c} \text{H} \quad \text{H}_2 \\ | \quad | \\ \text{OOC}-\text{C}-\text{N} \\ | \quad / \quad \backslash \\ \text{H}_2\text{C} \quad \text{S} \quad \text{Zn}/2 \end{array} \quad \text{と}$$

推定した。微酸性または中性の反応系で形成されたものには

$$\begin{array}{c} \text{H} \quad \text{H}_2 \\ | \quad | \\ \text{HS}-\text{H}_2\text{C}-\text{C}-\text{N} \\ | \quad / \quad \backslash \\ \text{O}=\text{C}-\text{O} \quad \text{Zn}/2 \end{array} \quad \text{の式を考えた。}$$

この他に両性イオンの形も考えたが，解離定数から考えて，その可能性はわずかであるとした。強酸性の反応系においては錯体は検出されなかった。以上の結果に基づいて $Zn - CySH (1 : 2)$ 錯体の調製法を検討し，83%の収量で白色ゲル状の標品を得た。