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Spectroscopic Investigation of Zn(II)-Poly(γ-glutamic acid) Complex in Aqueous Solution

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Abstract

Recently, naturally occurring biopolymers have been attracted considerable interest to synthesize metal-biopolymer complex as they are promising candidate for various biological applications. In this study zinc(II)-poly(γ-glutamic acid), [Zn(γ-pga)], complex has been synthesized in aqueous solution at different pH. The [Zn(γ-pga)] complex was characterized by uv-visible, infrared and nuclear magnetic resonance spectroscopic methods. From the analysis of spectroscopic data, the [Zn(γ-pga)] complex did not form at low pH. The suitable pH for complexation was found to be 4–5 and $Zn(OH)_2$ was produced at pH 7. The binding constant of Zn(II) ion with γ-pga was studied using equilibrium dialysis method. The binding constant of [Zn(γ-pga)] complex was found to be 0.94 L/mmol at pH 5 and 30 ^{o}C.

Keywords: Poly(γ-glutamic acid), zinc(II) ion, zinc(II)-poly(γ-glutamic acid) complex, binding constant

Introduction

Poly(γ-glutamic acid), γ-pga, is a water-soluble unusual anaionic homopolyamide that is made of D- and L-glutamic acid units connected by amide linkages between α amino and γ-carboxylic acid (Figure 1). The γ-pga was first discovered by Ivanovics and his co-workers as a capsule of *Bacillus anthracis* which was released into the medium upon autoclaving or upon aging and autolysis of the cell [1]. It is also well known that γ-pga is a component of Japanese traditional food natto, in other words it is an edible natural product which is very safe in the meaning of toxicity [2,3]. Therefore, this biopolymer has been attracted in the various fields such as food, water treatment, and in medicinal chemistry as a carrier of drugs [4–7]. However, the structure and the binding constant of metal-(γ-pga) complex, and its medicinal activity have not yet been studied extensively. Previously, we found that zinc(II) ion coordinated with γ-pga to form [Zn(γ-pga)] complex in aqueous solution, which exhibited a potent insulin mimetic activity [8]. This

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important finding prompted us to investigate the structural details and the binding constant of [Zn(γ-pga)] complex in aqueous solution. For this reason, it seemed worthwhile to study the complexation reaction in aqueous solution at various pH which would give an insight the structure of [Zn(γ-pga)] complex.

In this paper, the complexation reaction of zinc(II) ion with γ-pga has been investigated in aqueous solution (pH 2–7) using UV-visible, infrared and nuclear magnetic resonance (NMR) spectroscopic methods and the binding constant of [Zn(γ-pga)] complex was estimated in aqueous solution at pH 5.

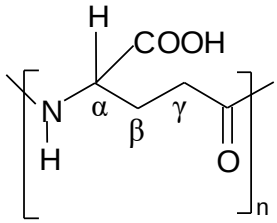

Figure 1: Structure of poly (γ-glutamic acid) (γ-pga).

Experimental

Materials

Zinc sulphate ($ZnSO_4.7H_2O$) was obtained from Wako Pure Chemical Industries (Osaka, Japan). A sample of γ-pga with a D/L-enantiomeric mixture having the average molecular weight 5.0×10^5 Da was obtained from BioLeaders Japan Corporation (Osaka, Japan). The polymer was used without further purification. Deionized water was prepared by passing distilled water through a deionizing column (Branstead, Syboron Corporation, Boston, USA). All other reagents were commercially available in the highest grade of purity and were used without further purification. The solution of γ-pga was prepared in aqueous or in duterium oxide solution by adding micro liters amount of 5 M NaOH or NaOD depending on experimental condition.

Spectroscopic measurements

UV-visible absorption spectra were recorded with Shimadzu UV-1601PC spectrophotometer, equipped with an electronically thermostated cell

holder (Shimadzu), in a quartz cell of path length of 1.0 cm. Before each measurement, the base line of spectrophotometer was calibrated against of respective solution. The pH of the reaction mixtures was determined by using HORIBA pH meter M-12 (Japan). The pH of the samples was adjusted by adding micro liter quantities of 5 M NaOH or 5 M HCl.

The Fourier transformation infrared (FTIR) spectra of γ-pga, $ZnSO_4$ and [Zn(γ-pga)] complex were measured in compressed KBr discs using FTIR spectrometer (IRPrestige-21 FTIR Spectrophotometer, Shimadzu, Japan) in the frequency range 400–4000 cm^{-1}.

For NMR measurements γ-pga and $ZnSO_4$ were dissolved in D_2O. Aliquots of 1 M $ZnSO_4$ solution were added successively to 1% γ-pga sample and the final concentration of $ZnSO_4$ was 10 mM. NMR measurements were carried with a NMR spectrometer (Varian UNITY INOVA 400NB) at 400 MHz. The ^{13}C chemical shifts in parts per million (ppm) were recorded downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal reference. A small amount of 5 M NaOD or 5 M DCl in the D_2O solution was used for the adjustment of pH 5. The values of pH here are direct meter readings of Horiba pH-meter without correction of deuterium effects.

Preparation of [Zn(γ-pga)] complex in solution

The [Zn(γ-pga)] complex was prepared in aqueous solution at various pH by mixing $ZnSO_4$ (1 M) with γ-pga (0.1–1% w/v) solutions. The final concentration of $ZnSO_4$ was 0.1–10 mM and that of γ-pga was 0.1–1% w/v depending on the measurement conditions. The pH of the samples was adjusted as described above.

Preparation of [Zn(γ-pga)] complex in solid state

The [Zn(γ-pga)] complex was prepared by mixing excess amounts of $ZnSO_4$ (1 M) and 10 ml of γ-pga (1%) solutions under refluxing at 80 ºC temperature for 4 h. After the reaction, a large amount of acetone was added to the reaction mixture and polymeric complex was precipitated. The precipitated complex was filtered, washed with acetone and deionzed water to remove excess amount of zinc. The resulting precipitate was dried on silica gel under a vacuum condition at room temperature.

Binding Equilibrium

The dialysis membranes were 10-cm strips of dialysis tubing (Spectra/Por molecularporous membrane tubing, Spectrum Laboratories, Inc, Rancho Dominguez, CA 90220-6435, USA; Size: flat width: 45 mm, diameter: 29 mm vol/length: 6.4 ml/cm) with a molecular weight cut-off not greater than 12-14000 Daltons. These membranes were prepared by immersing the membranes in hot water at 70 ^{o}C. At every one-hour interval the water was changed by fresh deionized water for efficient removal of sulfur and other soluble materials. These membranes were then stirred for one hour to bring them at room temperature. Stirring was continued with 70% methanol for 30 minutes. The membranes were then stored overnight in 50% methanol, rinsed with distilled water and soaked in a solution of desired pH for 2-3 hours [9]. Finally these were put into a solution of desired pH and stored in a refrigerator.

During batch experimentation a number of membranes were taken, one end of each knotted and then was filled with 3 mL of γ-pga (0.1% w/v) solution. The other end of each of the membranes was tied by nylon thread. All of them were placed in the 122 mL stoppered bottles containing 60 mL of zinc(II) solutions (0.1 − 10 mmol/L). The bottles were then placed in the water-bath shaking incubator and shaken at a speed of 120 r/min at constant temperature (30 ± 0.2 °C). The concentration of zinc in the outer solution was determined by atomic absorption spectrometer (AA-7000, Shimadzu Corporation, Japan) equipped with auto sampler ASC-7000.

A control experiment was also set up which contained only 3 mL of solvent inside the dialysis membrane and placed in the 122 mL stoppered bottle containing 60 mL of zinc solution. No interaction was found between membrane and zinc(II) The amount of zinc bound to γ-pga at equilibrium time t, q_e (mmol/g) was determined by

$$q_e = V(C_0 - C_e)/m \quad (1)$$

where C_0 and C_e (mmol/L) are the outer-phase concentrations of zinc(II) at initial and equilibrium time t, respectively; V is the total volume of the solution (L) and m is the amount of dry γ-pga used (g).

Results and discussion

Characterization of [Zn(γ-pga)] complex in aqueous solution by UV-visible spectroscopy

The absorption maxima of [Zn(γ-pga)] complex in aqueous solution at pH 5 appeared at 258 and 330 nm, whereas free γ-pga absorbed at 265 nm and $ZnSO_4$ showed no absorption band (Figure 2). Thus the blue shift (7 nm) of the absorption band at 258 nm and a new band around at 330 nm in the UV region must be ascribed to the transition of electrons associated with the complex formation. Similar phenomena was observed in the case of Zn(II) complex with functionalized polystyrene containing salicylaldehyde end group [10]. The band at 350 nm disappeared when Zn(II) coordinated with ligand and a new band appeared at 410 nm due to the charge transfer between central zinc atom and coordinated atoms.

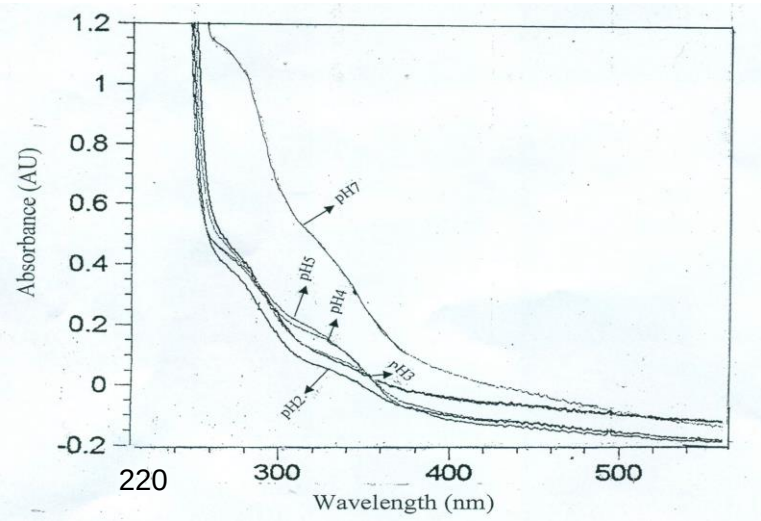

Figure 2: UV spectra of [Zn(γ-pga)] complex in aqueous solution at different pH.

In order to investigate the effect of pH on the complexation of zinc(II) ion with γ-pga in aqueous solution, the absorption spectra of γ-pga were measured in aqueous solution at pH 2–7 and the absorption maxima (λ_{max}) was found to be at 265 nm in these pH ranges. These spectra were compared with the spectra of [Zn(γ-pga)] complex taken in aqueous solution at the same pH values. At pH 2–3, no significant change was observed between the

spectra of γ-pga and [Zn(γ-pga)], indicating week interaction between zinc(II) ion and γ-pga. At pH 4−5, the spectra of [Zn(γ-pga)] showed a new band at 330 nm that may be assigned to charge transfer between zinc(II) ions and γ-pga. Again it was noticed the formation of gum like insoluble complex when excess amount of $ZnSO_4$ was added to γ-pga solution at this pH range. This new band disappeared with increasing solution pH and the band shifted to shorter wavelength near 265 nm, which is almost similar to the spectrum of free γ-pga. Thus the shift of absorption maximum of the complex in the UV region might be either changed in co-ordination mode zinc(II) ion or due to the formation $Zn(OH)_2$, where it does not deposit but remains in solution by hydrophobic interaction. On reaching pH 9, a copious quantity of white precipitate was found due to formation of $Zn(OH)_2$. Similar phenomena was observed in the case of Zn complex with poly(α-L-glutamic acid), [Zn(α-pga)] in aqueous solution studied by polarographic and circular dichroism spectroscopic methods [11].

The author concluded that Zn(II) ions do not bind with α-pga in aqueous solution at pH lower than 3. When pH increases from 5.5 to 7.0, the interaction between α-pga and Zn(II) ions was found more effective and finally $Zn(OH)_2$ was formed above pH 7. Thus the complexation reaction between zinc(II) ion and γ-pga is favourable in aqueous solution at pH 4–6.

Characterization of [Zn(γ-pga)] complex by FTIR spectroscopy

The FTIR spectrum of γ-pga exhibited the following absorption bands: the strong N–H stretching band at 3290 cm^{-1}, the strong stretching vibration band due to the C=O in the –COOH group at 1734 cm^{-1}, the bands at the region 1610–1650 cm^{-1} for amide I (C=O stretch) and 1545–1560 cm^{-1} for amide II (N–H bend) (Table 1). In the FTIR spectra of solid [Zn(γ-pga)] complex prepared at pH 5, the carboxylic group of γ-pga is involved in complexation reaction as confirmed by disappearance of band intensity at 1734 cm^{-1} (Table 1). In order to know the pH dependency of complexation reaction, the solid [Zn(γ-pga)] complex was prepared at different solution pH. The intensity of C=O stretching band appeared at 1734 cm^{-1} in FTIR spectrum of [Zn(γ-pga)] complex prepared at pH 3 was decreased but still remained. It may be due to the existence of partially protonated carboxylic acid groups that might not be deprotonated and/or coordinated to the metal center. This band position almost disappeared when the complex was

prepared at pH 4–6. These results supported that the oxygen atom of the side chain carboxylic group of γ-pga binds to zinc(II) ion. Kurotu also pointed out the involvement of carboxylic groups in case of [Zn(α-pga)] complex at pH 6.5 [11].

Table 1: FTIR absorption bands of γ-pga and [Zn(γ-pga)] complex taken in KBr

Sample	FTIR data in KBr (cm^{-1})
γ-pga	3290 (ν_{N-H} stretching band) 1734 ($\nu_{C=O}$ stretching band in –COOH) 1610–1650 ($\nu_{amide\ I\ C=O}$ stretching band) 1545–1560 ($\nu_{amide\ II\ N-H}$ bending band)
[Zn(γ-pga)]	The intensity of the band at 1734 cm^{-1} decreases with increasing pH, 1640 (convoluted $\nu_{amide\ I}$ and asymmetric ν_{COO^-} valency vibration band) 1404 (symmetric valency vibration band)

Characterization of [Zn(γ-pga)] complex by NMR spectroscopy

The [Zn(γ-pga)] complex was prepared in D_2O (pH 5) by mixing $ZnSO_4$ (1 M) and 1% of γ-pga solutions. The complex was characterized by ^{13}C NMR. Figure 3 shows the ^{13}C NMR spectra of γ-pga and [Zn(γ-pga)] complex. The ^{13}C NMR spectrum of γ-pga (Figure 3a) showed five typical signals at 178 ppm for carboxyl carbon, 181 ppm for carbonyl carbon, 57 ppm for α-C, 30 ppm for β-C, and 35 ppm for γ-C, respectively.

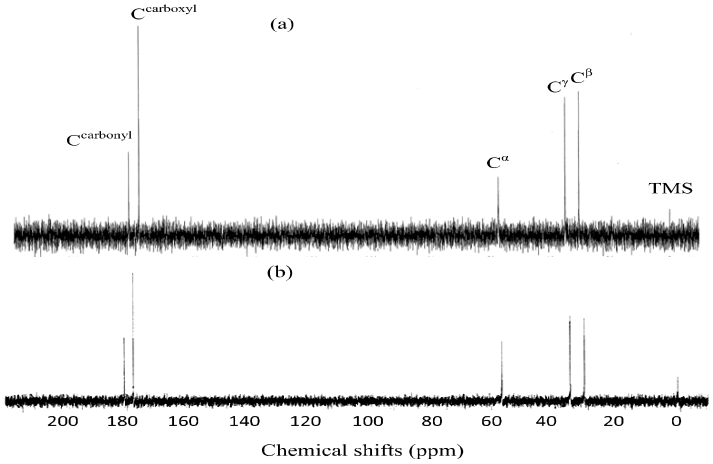

Figure 3: ^{13}C NMR spectra of γ-pga (a) and [Zn(γ-pga)] complex (b) in D_2O. The ^{13}C spectra were recorded downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal reference.

On adding $ZnSO_4$, the selective line broadening was observed and the signal intensity of the carboxyl carbon was greatly reduced (Figure 3b), indicating the formation of [Zn(γ-pga)] complex in which the oxygen atom of the side chain carboxyl group of γ-pga was bound to Zn(II) ion. Similar spectral phenomena were observed in the case of Cu(II)-Poly(D-glutamic acid) complex in aqueous solution, where Cu(II) ion specifically interacted with carboxyl group of the side chain of poly(D-glutamic acid) [12].

Binding equilibrium

The binding constant of [Zn(γ-pga)] complex was determined in aqueous solution at pH 5. The equilibrium amount of zinc bound to γ-pga (q_e; mmol/g) was plotted against equilibrium concentration of zinc (C_e; mmol/L) in Figure 4a. The experimental data was fitted by Langmuir model presented in Eqs. (2 and 3):

$$q_e = \frac{K_L}{(1 + a} \tag{2}$$

$$C_e/q_e = 1/K_L + (a_L/K_L)C_e \tag{3}$$

where q_e and C_e are defined as before and K_L (L/g) and a_L (L/mmol) are the characteristics of Langmuir equation and can be determined from intercept and slope of the linear plot of C_e/q_e vs. C_e (Figure 4b). The ratio of K_L/a_L gives the theoretical monolayer saturation capacity, q_m (μmol/g). The values of K_L, a_L and q_m were estimated to be 16.13 L/g, 0.94 L/mmol and 17.24 mmol/g, respectively.

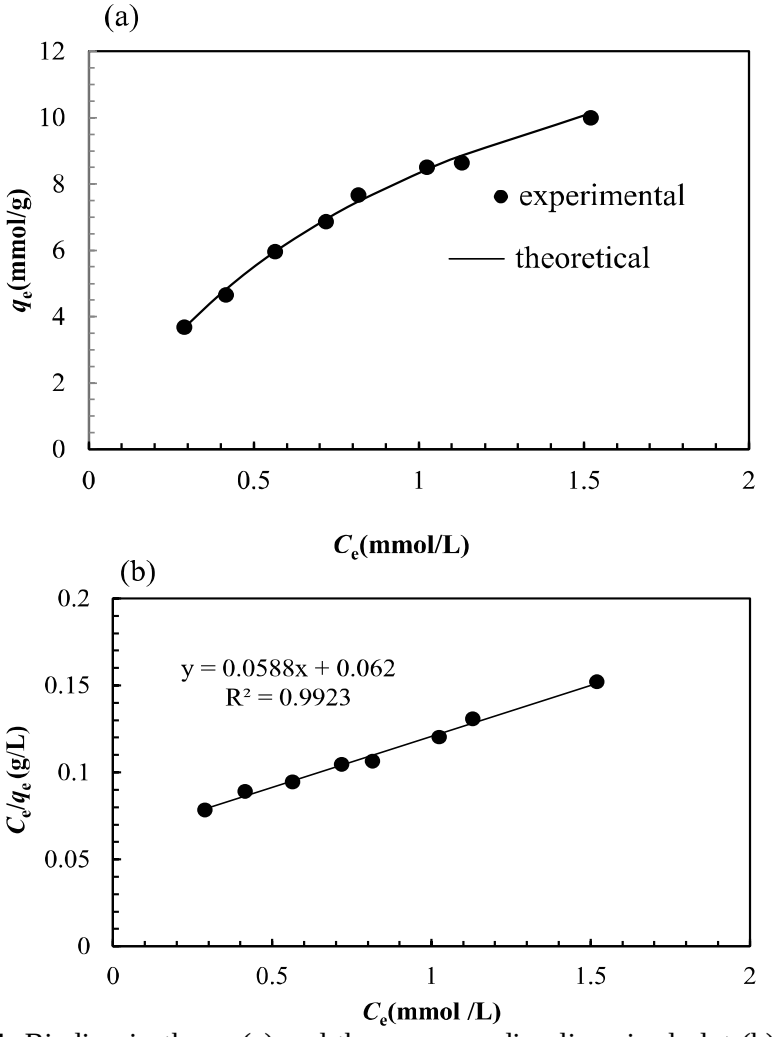

Figure 4: Binding isotherm (a) and the corresponding linearized plot (b) for the chelation between Zn(II) ions and γ-pga at pH 5 and temperature 30 °C. The theoretical solid line in Figure 4(a) was generated by using the Langmuir model equation (2) and the values of a_L (0.94 L/mmol) and K_L (16.13 L/g) obtained from Figure 4(b).

Conclusions

In conclusion, γ-pga is a naturally occurring biodegradable and edible polymer that is readily chelated with Zn(II) metal ions to form [Zn(γ-pga)]

complex. From the analysis of spectroscopic data it is confirmed that Zn(II) ion does not bind to γ-pga at pH 2−3. When pH increases from 4 to 5, the interaction between Zn(II) ions and γ-pga was significant and finally $Zn(OH)_2$ was formed at pH 7. Thus the binding constant of [Zn(γ-pga)] complex was estimated to be 0.94 L/mmol at pH 5 and 30 ^oC.

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