Equilibrium Dialysis Study of the Interaction between Poly(α-L-glutamic acid) and Transition Metal Ions in the Helix–Coil Transition Region [†]

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ABSTRACT: By the combined equilibrium dialysis-colorimetric method, the fractions, f_b, of bound Ni(II) and Cd(II) in the poly(α-L-glutamic acid)–Ni(II) and –Cd(II) solutions were determined at 25°C over a pH range of 7—4.2. Both Ni^{2+} and Cd^{2+} ions were dissociated by about 25% at pH 6.5 ($f_b \approx 75\%$), where poly(Glu) is in the random–coil form, and by nearly 90% at pH 4.5, where poly(Glu) is almost a complete helix. The ORD data of poly(Glu)–Ni(II) and –Cd(II) complexes were essentially the same as those of poly(Glu), indicating that these transition metal ions do not induce the helical structure in poly(Glu). This is a striking contrast with the case of Cu^{2+}. From the equilibrium dialysis data, it was concluded that each Ni^{2+} or Cd^{2+} ion binds with two adjacent glutamyl residues in poly(Glu)–Ni(II) and –Cd(II) complexes. The stability constants of these complexes were estimated to be of the order of 10^3.

   KEY WORDS   Poly(α-L-glutamic acid) / Transition Metal Ions / Equilibrium
Dialysis / Helix–Coil Transition / Complex Formation /

Interactions between poly(α-L-glutamic acid), poly(Glu), and a number of transition metal ions have been widely studied by means of optical, magnetic, and potentiometric titrations.[2–15] An absorption and circular dichroism study has shown that the helical structure is induced in poly(Glu) by bound Cu(II) in a pH range where the polyion is ordinarily in the random-coil form.[12] On the other hand, it has been reported that the Mn^{2+} ion binds to poly(Glu) but does not affect the pH-induced helix–coil transition.[11] These previous studies thus suggest that the interaction between poly(Glu) and a transition metal ion is rather specific, depending on the particular ionic species and also that the Cu^{2+} ion may be unique in forming multivariant complexes with Glu residues.

Equilibrium dialysis studies of poly(Glu)–Cu(II)[15] and polyacrylate–Cu(II)[16] have shown that the Cu(II) can bind to two to four side-chain carboxylato groups, either distant or adjacent, in these polyanions and that the bound Cu(II) tends to dissociate from the polymer residues with a large increase in proton concentration. In order to clarify the effect of metal ions on the conformational change of poly(Glu) quantitatively, the concentration of the metal ions actually bound to the polymer must be evaluated. Although equilibrium dialysis is a time-consuming method, it is a direct technique for determining the fraction of bound ions in a polyion–metal ion system.

In this work, interactions between poly(Glu) and Ni^{2+}, Cd^{2+}, and Cu^{2+} ions are presented, with emphasis on the pH dependence of the amount of bound ions, the scheme of binding reactions, and the helix-inducing ability of these bound ions. The results from equilibrium dialysis and ORD clearly indicated that both Ni(II) and Cd(II) are bound to poly(Glu) to an extent considerably less than Cu(II) and that their effect on the conformational change of poly(Glu) is almost negligible.

EXPERIMENTAL

Materials

The sodium salt of poly(Glu) was the same sample as used in a previous work.[12] The metal salts,

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[†]   This is Macromolecule–Metal Ion Complex. VI.[1]

NiCl₂·6H₂O, CdCl₂·2.5H₂O, and CuCl₂·2H₂O, and other chemicals were of reagent grade and used without further purification.

Preparation of Poly(Glu)–Metal Ion Solutions

In a poly(Glu)–metal ion solution, the residue concentration of poly(Glu) was 8 mmol dm⁻³, while the sum of the concentrations of the added HCl and NaCl was maintained to be 150 mmol dm⁻³ throughout the pH range to avoid the Donnan effect.[15,16] The mixing ratio, R, which was constant at 16, was defined as the mean residue weight concentration of Glu residues divided by the total concentration of metal ions in the sample solution.

Methods and Measurements

Equilibrium dialysis, photometric, and pH measurements are all described elsewhere in detail.[15,16] Quantitative determination of unbound Ni²⁺ and Cd²⁺ in the dialyzed solutions was carried out colorimetrically; the dimethylglyoxime method for Ni²⁺[17,18] and the dithizone method for Cd²⁺[17,19,20] The fraction of unbound metal ions in the poly(Glu)–metal ion solution, $1-f_b$, is defined as,

$$1-f_b=\frac{[M^{2+}]_f}{[M^{2+}]_b+[M^{2+}]_f} \qquad (1)$$

where $[M^{2+}]_f$ and $[M^{2+}]_b$ are the concentrations of unbound and bound metal ion M^{2+}. The ORD curves were measured on a JASCO ORD/UV-5 spectropolarimeter in the 350–220 nm region. The mean residue rotation of Glu residues, $[m]$, was defined as $[m]=151[\alpha]/100$, where $[\alpha]$ is the specific rotation. All measurements were performed at $25\pm2°C$.

RESULTS AND DISCUSSION

The pH Dependence of the Fraction of Bound Ni(II), Cd(II), and Cu(II)

In Figure 1, the fractions of unbound metal ions obtained from equilibrium dialysis are plotted against pH for three poly(Glu)–metal ion systems. The Ni²⁺ and Cd²⁺ ions are bound to poly(Glu) rather weakly even in a high pH range (>6). About 25% of these ions are already dissociated at pH 6.5, while one-half of the Ni²⁺ and Cd²⁺ ions in solutions remain unbound in the incipience of the coil-to-helix

transition near pH 5.5. Almost 90% of Ni²⁺ and Cd²⁺ ions are dissociated, when the helix of poly(Glu) is completed near pH 4.5. Contrary to the poly(Glu)–Ni(II) or –Cd(II) system, the dissociation of Cu(II) from the poly(Glu) site becomes appreciable only after completion of the helical structure with a further decrease in pH.[15] It is now clear that the metal ions, Ni²⁺, Cd²⁺, and Cu²⁺, bind to the charged poly(Glu) in the extended coil, but tend to dissociate from it in a lower pH range to a considerable degree.
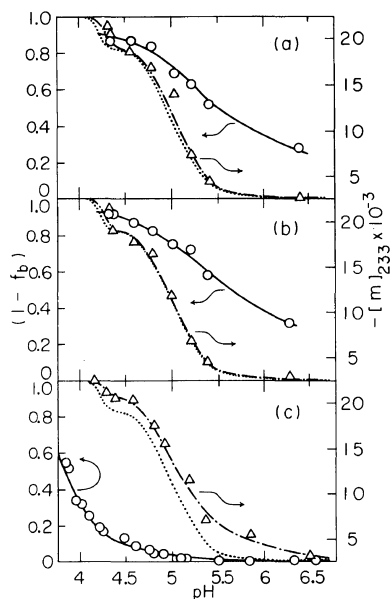
The pH Dependence of the Molar Rotation of Poly(Glu)–Metal Ion Solutions

As the variation in $[m]$ with pH indicates in Figure 1, the bound Cu(II) induces a helix in poly(Glu). This is particularly noticeable at the first stage of the conformational change of poly(Glu) from the coil to the helix (triangles in Figure 1). The coil-to-helix transition of poly(Glu) is, however, unaffected by the binding of the Ni²⁺ or Cd²⁺ ion to poly(Glu), since the transition curve of poly(Glu) essentially coincides with that of the poly(Glu)–metal ion solution. Thus, the conformation of poly(Glu) remains unchanged in the presence or the absence of the Ni²⁺ or Cd²⁺ ion throughout the pH range 7—4.2. The results in Figure 1 suggest that the tendency of dissociation of the metal ion is not necessarily related to the conformational change in the poly(Glu)–metal ion complex. The amount of unbound Ni²⁺ and Cd²⁺ ions seems related to the charge neutralization of the ionized side-chain carboxylates. On the other hand, most of Cu²⁺ ions remain bound in poly(Glu) even in the helical conformation.

In order to discuss the effect of bound metal ions on the coil-to-helix transition on the basis of the degree of dissociation of the side-chain carboxylato group, the $[m]$ values are replotted against the degree of dissociation $\alpha^{\bullet}$ in Figure 2. The $\alpha^{\bullet}$ is defined as,[16]

$$\alpha^{\bullet}=1-\frac{[H^+]_{added}+2[M^{2+}]_b}{[Glu]_o} \qquad (2)$$

when the carboxylato groups are partially neutralized by bound M^{2+} and H^+ ions at a given pH. Here $[Glu]_0$ is the concentration of Glu residues in solution. If a bound bivalent metal ion affects the coil-to-helix transition in the same manner as two bound protons, the pH-induced transition curve of poly(Glu) should be superimposed upon by the

Figure 1. The pH dependence of the fraction $(1 - f_b)$ of unbound metal ions, M^{2+}, (—○—) and the mean residue rotation $[m]$ at 233 nm of poly(Glu) in the presence (—·△—·) and absence (·····) of M^{2+}: (a) poly(Glu)–Ni(II); (b) poly(Glu)–Cd(II); (c) poly(Glu)–Cu(II). The $(1 - f_b)$ curve of poly(Glu)–Cu(II) system is taken from ref 15. The mixing ratio R is 16 in all cases. The contribution of the Cotton effect associated with a charge-transfer band of bound Cu(II) near 260 nm ($\varepsilon = 3000 \sim 5000$) to the $[m]_{233}$ value has been shown to be negligibly small as compared with the contribution from the peptide chromophore.[12] The poly(Glu)–Ni(II) sample exhibits a weak band near 394 nm ($\varepsilon \simeq 8$) in the 220—400 nm region. The optical rotatory power of this band should also be negligible under consideration of the value of the optical dissymmetry factor ($\Delta\varepsilon/\varepsilon \simeq 2 \times 10^{-4}$) of bound Cu(II).[12] In fact, the shapes of ORD curves of poly(Glu) underwent no change either in the presence or absence of Cu^{2+} or Ni^{2+} in the 230—300 nm region.

curves of poly(Glu)–metal ion systems. This is not the case, however, as is seen from Figure 2. From the incipience to the midpoint of the transition, a Cu^{2+} ion contributes to helix formation more than two protons as it binds to poly(Glu). In the same $\alpha^{\bullet}$ range of 0.8—0.5, however, a bound Ni^{2+} or Cd^{2+} ion is clearly much less effective than two bound protons in inducing a helix structure in poly(Glu). This is probably because a Ni^{2+} or Cd^{2+} ion binds to two carboxylato groups suppressing the ionization electrostatically, but the binding is in such a way as to
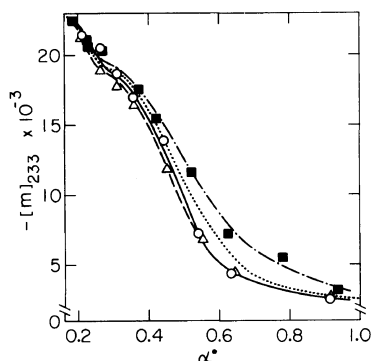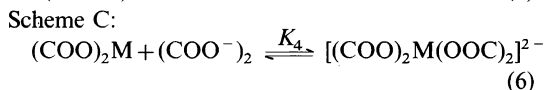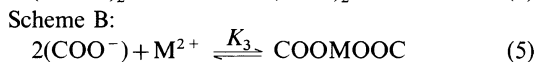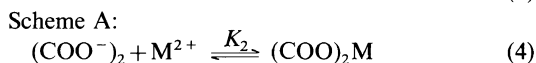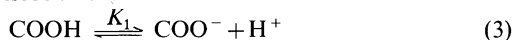

Figure 2. The dependence of the mean residue rotation, $[m]$, at 233 nm on the degree of dissociation of poly(Glu), $\alpha^{\bullet}$, in the absence of M^{2+} ions (·····) and in the presence of Ni^{2+} (—○—), Cd^{2+} (--△--), and Cu^{2+} (—·■—·).

retard, or remain indifferent to, the formation of an intrastrand helix. The Cu^{2+} ion interacts with Glu residues by chelation and coordination besides charge neutralization to stabilized helix fragments.[12,14,15]

Schemes of Binding between Poly(Glu) and Metal Ions

The above result suggests that the binding mechanism toward poly(Glu) may be specific to the ionic species. Just as shown for the poly(acrylic acid)– and poly(Glu)–Cu(II) systems,[15,16] three schemes (A, B, and C) are considered for the poly(Glu)–Ni(II) and –Cd(II) systems.

Dissociation:
$$COOH \xrightleftharpoons{K_1} COO^- + H^+ \qquad (3)$$
Scheme A:
$$(COO^-)_2 + M^{2+} \xrightleftharpoons{K_2} (COO)_2M \qquad (4)$$
Scheme B:
$$2(COO^-) + M^{2+} \xrightleftharpoons{K_3} COOMOOC \qquad (5)$$
Scheme C:
$$(COO)_2M + (COO^-)_2 \xrightleftharpoons{K_4} [(COO)_2M(OOC)_2]^{2-} \qquad (6)$$

An M^{2+} ion binds to either two adjacent side-chain carboxylates (Scheme A), any two carboxylates (Scheme B), or any two pairs of adjacent carboxylates (Scheme C). (The coordination of the –NH– group to Cu^{2+} was evident in the frozen state from ESR results.[3,14] If the same occurs at room temperature, this coordination may be considered as a

secondary and spontaneous reaction which does not depend on the concentration of Glu residues, since the electrostatic interaction between Cu^{2+} and COO^- is dominant.[15])

The equilibrium expressions for Schemes A, B, and C are written as

A: $\quad \log\left(\dfrac{q(P_o - 2\beta)K_2}{\sigma} - 1\right) = -pH + pK_1 \qquad (7)$

B: $\quad \log\left(\dfrac{(P_o - 2\beta)\sqrt{K_3}}{\sqrt{\sigma}} - 1\right) = -pH + pK_1 \qquad (8)$

C: $\quad \log\left(\dfrac{q(P_o - 4\beta)\sqrt{K_2 K_4}}{\sqrt{\sigma}} - 1\right) = -pH + pK_1$
$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad (9)$

where $P_0 \equiv [Glu]_0 = [COO^-] + [COOH] + n\beta$ ($n = 2$ for Schemes A and B, and $n = 4$ for Scheme C), $\beta = [M^{2+}]_b$, and $\sigma = [M^{2+}]_b/[M^{2+}]_f$. The apparent dissociation constant and stability constants are denoted by K_1 and $K_2 \sim K_4$, respectively. The concentration of the adjacent carboxylato groups was assumed to be one-half that of the total carboxylato groups ($q = 1/2$).[16] (Detailed discussion has been given in ref 16.)

The quantities of the left-hand side of eq 7 to 9 should yield a straight line, whose slope is -1, with respect to pH, if complex formation occurs according to particular Schemes A to C. The results are shown in Figure 3, where the equilibrium dialysis data of poly(Glu)–Ni(II) and –Cd(II) are tested to fit Scheme A, B, or C. Clearly the data of the Ni^{2+} and Cd^{2+} ions fit Scheme A quite well throughout the pH range 6.5—4.2. These experimental points, however, fail to fit both Schemes B and C, systematically deviating from the most probable straight lines whose slopes are -1.

Therefore, the formation of complex between Glu residue and the Ni^{2+} or Cd^{2+} ion is probably described reasonably well by Scheme A (but not by Scheme B or C) from which the constants $pK_1 = 5.9$ and $\log K_2 = 3.0$ were evaluated for the Ni(II)–Glu complex and $pK_1 = 5.9$ and $\log K_2 = 2.9$ for the Cd(II)–Glu complex. It should be noted that the data of Cu(II)–Glu complexes behave in a very complicated manner and do not fit any single Scheme A, B, or C over the entire pH range.[15]

In conclusion, each Ni^{2+} or Cd^{2+} ion probably binds to only two adjacent Glu residues of poly(Glu)
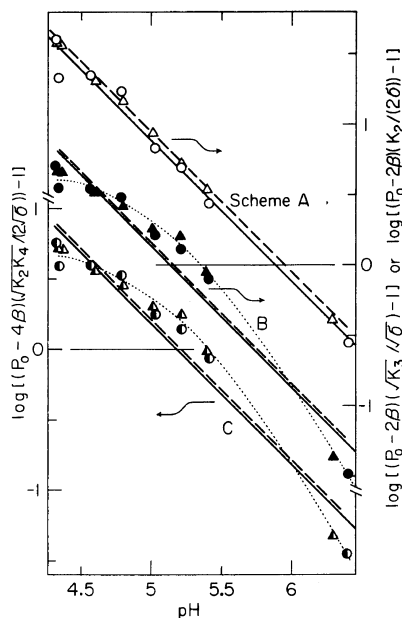
Figure 3. The stoichiometric analysis of the binding reactions for poly(Glu)–Ni(II) (circles) and poly-(Glu)–Cd(II) (triangles) systems. The data points fitted Schemes A (open symbols), B (filled ones), and C (half-filled ones). For each scheme, the best straight lines were obtained by the least-squares method (the solid lines for poly(Glu)–Ni(II) and the dashed lines for poly-(Glu)–Cd(II)). The dotted arcs show the systematic deviation of the data points.

and forms a Glu complex with a low stability constant as compared with the Cu(II)–Glu complex. These results seem compatible with the fact that the bound Ni(II) or Cd(II) do not contribute to the induction of the helical structure. Although time-consuming and lacking in versatility, equilibrium dialysis-colorimetric techniques are useful for determining the amount of unbound metal ions, and this is indispensable for quantitative studies of macroion–small ion interactions.

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