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Direct Current Polarographic and Circular Dichroism Spectroscopic Investigations on Helix–Coil Transition of Cd^{2+} –Poly(α -L-glutamic acid) Complex

Takuzo KUROTU and Motomu KASAGI

*Department of Chemistry, The National Defense Academy,
Yokosuka-shi, Kanagawa 239, Japan*

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Helix–coil transition of polypeptides is known to be induced by change in pH,¹ composition of solvent,² or the presence of metal ions.^{3,4} This transition especially in the presence of metal ions has been extensively investigated by many techniques,^{5–8} such as nuclear magnetic resonance, circular dichroism spectroscopies, and equilibrium dialysis method, since metallo-polypeptide complex may be considered as a usefull model for understanding the mode of action for metallo-proteins.

A very few polarographic studies,^{9,10} however, have been reported on the metallo-polypeptide complex, although polarography is a useful technique to investigate the behavior of metal ions that coordinate the polypeptide in solution. The polarographic diffusion current of metal ions such as Cu^{2+} , Zn^{2+} , Co^{2+} , or Ni^{2+} is known to decrease in the presence of proteins with increase in pH. This behavior was interpreted by Tanford in terms of the complex formation between metal ions and proteins.¹¹ Inoue *et al.*,¹⁰ however, suggested that his results could be explained more adequately in terms of the conformational transition mechanism in conjunction with the complex formation.

This paper concerned with the direct current polarographic behavior of the Cd^{2+} –poly(α -L-

glutamic acid) (PGA) complex in the helix–coil transition region. The results are discussed together with those of the circular dichroism (CD) spectra of the same solution. Cd^{2+} ion was employed as a ligand of PGA because Cd^{2+} is also known to form a helix structure with PGA and provides a well defined reduction wave.

Sodium poly(α -L-glutamic acid) was purchased from the Peptide Institute, Inc., (Osaka) (degree of polymerization, $\text{DP} = 175$). Cadmium sulfate and sodium perchlorate were of analytical grade (Wako Pure Chemical Industries). The pH values of all solutions were adjusted with a solution of 0.1 M HCl or 0.1 M NaOH. The value of $f(=[\text{PGA}]/[\text{Cd}^{2+}])$, defined as the concentration of PGA, were controlled by adding various amounts of PGA to a constant Cd^{2+} concentration which was kept to be 5×10^{-4} M in all measurements. Sodium perchlorate was employed as a supporting electrolyte at a concentration of 0.01 M.

Polarographic limiting currents were measured using a Yanagimoto Model P-8 Polarograph with a saturated calomel electrode as a reference electrode. The characteristics of the capillary employed were 0.836 mg s^{-1} as the flow rate and 1.28 s as the drop

time. The circular dichroism measurements were carried out on a Jasco J-500A spectropolarimeter (Japan Spectroscopic Co., Ltd.) equipped with a DP-500 data processor. pH was measured using a TOA Model HM-6A pH meter.

Effects of mercury column height on the limiting current for Cd^{2+} -PGA solution were studied. The limiting current for Cd^{2+} was found to be entirely diffusion controlled over the range of the mercury column height (50–80 cm) examined.

Figure 1 shows the effects of variation in pH on the diffusion current of the solutions containing 5×10^{-4} M cadmium. All well defined polarograms indicate the reversible reaction. As the pH value of a Cd^{2+} -PGA solution increases from 3.8 to 7.1, the diffusion current for Cd^{2+} decreases steadily and reaches a definite value. However, in the absence of PGA, it remains unchanged in the same pH region. Figure 2 shows the CD spectra of these solutions. In the neutral or alkaline pH region, PGA is known to be in a random coil state,¹ whose spectrum has a very weak negative band at 238 nm, a weak positive band at 215 nm, and a very strong negative band at 198 nm. In the acidic pH region, PGA is in a helix state,¹ whose spectrum has two negative bands at 222 nm and 207 nm, and a positive band at 195 nm. The CD spectra of all Cd^{2+} -PGA solutions at the pH range from 3.8 to 7.1 clearly show a helix pattern. This indicates that Cd^{2+} ion binds to PGA to form an α -helix complex in the neutral pH region. The results also suggest that the decrease in diffusion current is due to the formation of Cd^{2+} -PGA helical complex because its diffusion coefficient is much smaller than that of free Cd^{2+} . We also investigated the effect of pH variation on the diffusion currents for the solutions containing various amounts of PGA ($f=0.65$ –6.5). The results are shown in Figure 3. It can be seen that in the absence of PGA, the diffusion current remains unchanged in the pH range from 3.0 to 8.0,

Figure 1. The pH dependence of diffusion current (i_d) in Cd^{2+} -PGA solution at $f=0.65$.

Figure 2. The pH dependence of CD spectra for Cd^{2+} -PGA solution at $f=0.65$.

and then decreases when the precipitates of $\text{Cd}(\text{OH})_2$ appeared over pH 8.0.

The diffusion current at various f values begins to decrease at nearly pH 4.5 and then becomes constant over pH 6.0. In the neutral pH region it decreases with increasing f value. This behavior means that the amount of free Cd^{2+} ions decreases with increase in that of the Cd^{2+} ions bound to PGA in the solution. Therefore, it is possible to estimate the fraction of the helix in this pH range from the

Figure 3. The relationship between diffusion currents for Cd^{2+} and pH values for Cd^{2+} -PGA solutions at various values of f . $\circ$, $f=0$; $\odot$, $f=0.65$; $\bullet$, $f=0.98$; $\ominus$, $f=1.30$; $\oplus$, $f=1.95$; $\ominus$, $f=2.60$; $\bullet$, $f=3.25$; $\diamond$, $f=6.50$.

results shown in Figure 3, provided that all of the Cd^{2+} -PGA have the helix structure. The CD spectra were also measured for the same solutions. The magnitude of molecular ellipticity $[\theta]$ at 222 nm is regarded as an indicator of helix content.¹² Figure 4 shows the relationship between the molecular ellipticity at 222 nm and pH for the Cd^{2+} -PGA solution at various f values. The magnitudes of molecular ellipticity of the solutions in the absence and in the presence of PGA are the same in the pH range from *ca.* 4.0 to *ca.* 5.0, although in this pH range, the diffusion currents for the solutions with PGA are always smaller than that for the solution without PGA. This shows that Cd^{2+} remains bound to PGA even in the helix region at which the ionization of the carboxylate group is suppressed to maintain the helix structure by hydrogen bonding between NH and CO groups in the main chain. This fact is noteworthy and in agreement with ^{13}C NMR results by Hiraoki *et al.*^{5,13} on the transition metal ion-PGA complex. In the pH region over 5.0, the magnitude of $[\theta]$ at 222 nm increases as f value becomes small. As pH exceeds 5.0, H^+ ion in the carboxylate groups

Figure 4. The relationship between molecular ellipticity at 222 nm and pH values for Cd^{2+} -PGA solutions at various values of f . $\odot$, $f=0.65$; $\bullet$, $f=0.98$; $\ominus$, $f=1.30$; $\oplus$, $f=1.95$; $\ominus$, $f=2.60$; $\bullet$, $f=3.25$; $\diamond$, $f=6.50$.

is gradually replaced by Cd^{2+} ion. Masujima *et al.*¹⁴ explained that bridging of carboxylate group by the bound Cd^{2+} ions maintains the helix structure in this pH range. At over pH 6.0, the magnitude of $[\theta]$ at 222 nm without Cd^{2+} is already zero (no helix structure exists), while that with Cd^{2+} begins to increase with pH, especially at small f values ($f \leq 2.60$). In this pH region, the formation of a helix structure is almost dominated by Cd^{2+} ions, and the interaction between PGA and Cd^{2+} was observed to get more effective at small f values as the pH became large. This is because the probability of ionization for the neighboring carboxylate groups upon a Cd^{2+} bound one becomes high to form a helix easier with increase in pH. For large f values ($f=3.25, 6.50$), this probability also becomes high; however, the increase in helix content is undetectable in CD spectra be-

Figure 5. The relationship between helix content determined by circular dichroism and that by polarography at pH 7.0. $\circ$, $f=0.65$; $\bullet$, $f=0.98$; $\odot$, $f=1.30$; $\oplus$, $f=1.90$; $\bullet$, $f=2.60$; $\oplus$, $f=3.25$; $\oplus$, $f=6.50$.

cause of the high concentration of PGA to Cd^{2+} ion. The diffusion current at various f values is almost invariant in the pH range over 6.0. Therefore, only in this pH range, the decrease of the diffusion current is completely ascribed to the binding of Cd^{2+} to PGA to form a helix structure. The helix content of the Cd^{2+} -PGA solution can be calculated through change in the diffusion current of Cd^{2+} ion. This calculation, however, can be applicable only to the solution whose pH is over 6.0. Figure 5 shows the relationship between the fraction of helix, determined by circular dichroism, H_{CD} , and that by polarography, H_{p} , at pH 7.0. It is obvious that H_{CD} is equal to H_{p} within a few percent difference at several f values. Therefore, it is found that the diffusion current can be regarded as a useful tool for determining the helix content in addition to other conventional methods such as the CD or IR method. H_{CD} and H_{p} are defined as follows:

$$H_{\text{CD}} = \frac{[\theta]_{222 \text{ nm}}}{38000} \times 100$$

$$H_{\text{p}} = \frac{i_{\text{d},f} - i_{\text{d},r}}{i_{\text{d},f}} \times \frac{1}{f} \times 100$$

where $i_{\text{d},f}$ denotes the diffusion current for free Cd^{2+} , and $i_{\text{d},r}$, the reduced diffusion current due to the attachment of Cd^{2+} to PGA. In conclusion, the polarographic diffusion current is found to be a sensitive parameter to the formation of α -helix of the polypeptide which undergoes helix-coil transition in the presence of metal ions. It can also provide useful information on the interaction between metal ions and polypeptides and on the helix content in conjunction with circular dichroism.

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