Polarographic Investigation of Biopolymers. II. Cu(II)-poly (α, L-Glutamic Acid) in the Helix-Coil Transition Region[1]

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The pH-induced helix-coil transition of the complex of Cu(II)-poly(α,L-glutamic acid) was studied with polarographic method. The diffusion current was found to change very sharply in a narrow range of pH and this change depended on the amount of Cu(II) ions. The half-wave potential of the complex was also dependent on pH and mixing ratio. The effect of Cu(II) ions on the secondary structure of the polymer was discussed from the viewpoint that the helix-coil transition of PGA is responsible for the change of the mode of binding interaction between Cu(II) ions and PGA sites. Based on the data of diffusion currents *versus* mixing ratios, the helical fractions of the complex at pH 3.4 and 4.0 were evaluated to be 0.37 and 0.11, respectively. From a value of the diffusion coefficient of the complex at pH 5.0, the molecular size in a random-coil form was calculated to be approximately 70 Å in diameter. The coordination number of the complex in the coiled form depended on the mixing ratio; the number was 3.4 in excess of polymer and 0.2—0.3 for the mixing ratio between 3.2 and 13. These values were discussed in terms of the possible mechanism of coordination. Finally, the activation energy of diffusion was obtained for complexes in helical and random-coil forms.

Biopolymers such as nucleic acids and proteins have been known to form specific and/or non-specific complexes with many physiologically important metallic ions. These complexes have been demonstrated to play significant roles in enzymatic or photosynthetic reactions *in vivo*. Towards the ultimate understanding of this complicated biological function, the elucidation of the mechanism of such complex formation *in vitro* has been attempted by means of various physico-chemical methods such as spectroscopy,[2] optical rotatory dispersion,[3] magnetic resonances,[4] potentiometric titration,[5] and polarography.[6]

Synthetic water-soluble homopolyamino acids have usually been employed, as the simplest model compound of natural proteins, to study the effects of secondary structures and conformational transitions upon the complex formation with metallic ions.[7] Among these polymers, the most frequently used one is poly(α,L-glutamic acid), PGA, which has ionizable carboxyl side chain groups and can undergo pH-induced helix-coil transitions. This polymer as such and complexes with metallic ions have also been investigated with aforementioned methods; however, very little has been unraveled regarding the ionic processes of the ligands (*i.e.*, polymer residues) from electrochemical points of view. Recently, some polarographic data of Cu(II)–PGA in the pH-induced helix-coil transition region were provided for the first time.[6]

Besides the numerous applications to metal–amino acids complex formation,[8] polarographic methods are useful in the study of (1) biopolymers which have intrinsic groups reducible at the dropping mercury electrode and (2) those to which some reducible metallic ions are attached extrinsically as a detecting probe. Earlier, Tanford reported that the polarographic limiting current of metallic ions decreased with increase in pH in the presence of bovine serum albumin.[9] He also noted that this observation was due to some complex formation between the protein and metallic ions. However, he was unable to elaborate his result in terms of the conformational transition of the protein. Saroff and Mark[10] also found similar results in the study of serum albumin in the presence of mercury or zinc. Rao and Lal[11] studied the complex formation of zinc and cadmium with native and chemically modified bovine serum albumin. Malik and his associates studied the interaction of copper[12] and lead[13] with transfusion gelatin. Recently, Malik and Jindal[14] reported the binding of copper(II) and cadmium with ovalbumin. Although these proteins are known to have a large fraction of helix,[15] none of these workers has attempted to correlate the secondary structure and the ability of binding to the metallic ions.

In the preliminary communication,[6] we have shown

1) The first of this series is Ref. 6.

2) G. Felsenfeld and S. Huang, *Biochim. Biophys. Acta*, **37**, 425 (1960); R. J. P. Williams, *Biopolymers, Symposia*, No. **1**, 515 (1964); J. Peisach, P. Aisen, and W. E. Blumberg, Ed., "The Biochemistry of Copper", Academic Press Inc., New York, N. Y., (1966).

3) D. D. Ulmer and B. L. Vallee, *J. Biol. Chem.*, **236**, 730 (1961); A. J. Adler, G. D. Fasman, and M. Tal, *Biochim. Biophys. Acta*, **213**, 424 (1970); M. Nakai, M. Yoneyama, and M. Hatano, This Bulletin, **44**, 874 (1971).

4) J. Ferretti, *Chem. Commun.*, **1967**, 1030; E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Nature*, **216**, 862 (1967); J. L. Markley, D. H. Meadows, and O. Jardetzky, *J. Mol. Biol.*, **27**, 25 (1967).

5) A. Wada, *Mol. Phys.*, **3**, 409 (1960); M. Nagasawa and A. Holtzer, *J. Amer. Chem. Soc.*, **86**, 538 (1964).

6) S. Inoue, K. Yamaoka, and M. Miura, This Bulletin, **44**, 1443 (1971).

7) H. Takesada, H. Yamazaki, and A. Wada, *Biopolymers*, **4**, 713 (1966).

8) M. Kodama and S. Takahashi, This Bulletin, **44**, 679 (1971); T. Nozaki, M. Kadowaki, D. Sagawa, and K. Orita, *Nippon Kagaku Zasshi*, **88**, 1168 (1967); **91**, 64 (1970).

9) C. Tanford, *J. Amer. Chem. Soc.*, **74**, 211 (1952).

10) H. A. Saroff and H. J. Mark, *ibid.*, **75**, 1420 (1953).

11) M. S. Narsinga Rao and Hira Lal, *ibid.*, **80**, 3222 (1958).

12) W. U. Malik and Salahuddin, *J. Electroanal. Chem.*, **5**, 147 (1963).

13) W. U. Malik and M. Muzaffaruddin, *ibid.*, **6**, 214 (1963).

14) W. U. Malik and M. R. Jindal, *ibid.*, **19**, 436 (1968).

15) E. R. Blout "Optical Rotatory Dispersion," ed. by C. Djerassi, McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 272; E. Shechter and E. R. Blout, *Proc. Natl. Acad. Sci. (US)*, **51**, 794 (1964).

that the polarographic diffusion current of the Cu(II)–PGA complex changes with pH in the region between 3.6 and 6.0 and we noted that this change parallels the pH-induced helix-coil transition of PGA. In this paper, we present a further quantitative and detailed work on the pH-induced helix-coil transition of the Cu(II)–PGA complex in aqueous solutions. Our emphasis will lie on the following points: (1) the contribution of the Cu(II) ion to the conformational transition of PGA which can be established from the study of the effect of pH on the diffusion current, $(i_d)_p$, at various molar mixing ratios, R, of glutamic acid residue to Cu(II) ion, (2) the bonding models of Cu(II)–PGA complex which can be estimated from the relation between half-wave potential, $E_{1/2}$, and mixing ratio at various pH's, and (3) the interaction of Cu(II) ions with PGA in either helical or random-coil conformation which can be evaluated from the activation energy of diffusion calculated from the data of temperature effect on diffusion current of Cu(II)–PGA.

Experimental

Materials. PGA, which is in the form of sodium salt, was supplied from Ajinomoto Co., Inc. and used without further purification. An average degree of polymerization of 700-800 was estimated from the intrinsic viscosity in 2M sodium chloride solution at pH 7.0. The mean residue weight of sodium polyglutamate was taken to be 169.[16] Cupric chloride, sodium chloride, and hydrochloric acid were all of analytical grade.

Preparation of sample solutions. In order to maintain the ionic strength of the Cu(II)–PGA complex solution throughout this work at a constant level of 7.5×10^{-3} (which is equal to the final concentration of chloride ions in solution), the sample solution was prepared in such a way that a desired pH value was obtained by adding both hydrochloric acid and sodium chloride solution (which was employed as supporting electrolyte) in appropriate proportions. A stock PGA solution of 1.5 g/100 m*l* or 8×10^{-2}M was diluted prior to measurements. The molar mixing ratios, R, were controlled by varying the concentration of PGA at a constant Cu(II) concentration which was kept to be 5×10^{-4}M in all measurements.

Polarographic measurements. Polarographic limiting currents were measured with a Yanagimoto Model P-8 polarograph. Each current-potential curve was manually obtained by plotting the current at intervals of 10 mV potentials. Since the removal of dissolved oxygen is essential but the bubbling nitrogen gas through a sample solution at low pH causes a separation of fibrous Cu(II)–PGA, it was necessary to degas a hydrochloric acid-containing Cu(II) solution in a glass vessel, which has a narrow outlet and a gas inlet capillary, and a PGA solution in a polarographic cell individually before mixing. These two solutions were then mixed very slowly to avoid precipitation. The mixing order was always a Cu(II) solution to a PGA solution. With this method of mixing the sample solution was successfully prepared at a pH as low as 2.7. After 20 min of degassing, flushing of nitrogen gas was continued while current-potential plots were recorded. The value of $E_{1/2}$ was determined by plotting $\log[i/(i_d-i)]$ against dropping mercury electrode potential. Thus the intercept at $\log[i/(i_d-i)]=0$ yields $E_{1/2}$ and the slope of the plot is a measure of reversibility (see Discussion). A glass capillary, which has a flow rate of 1.0 mg/sec and a drop time of 8.0 sec, was used as dropping mercury electrode.

Verification of polarographic diffusion current. The polarographic limiting currents were measured at four different heights of a mercury column for both helical and coiled Cu(II)–PGA complex solutions. A strictly linear curve was obtained for each solution when the limiting current was plotted against the square root of the height of mercury column. These curves could be extrapolated to intercept the origin for both solutions. Therefore, the limiting current of Cu(II)–PGA solutions at such a low supporting electrolyte concentration as 7.5×10^{-3}M could be confirmed to be diffusion current.

Temperature was kept at 25±0.05°C except when the dependence of diffusion current on temperature was studied.

pH measurements were carried out with a Toa Electronics Model HM-5A pH-meter.

Results

Current-Potential Curves with Variation of pH. Current-potential curves obtained in the pH region between 3.0 and 6.0 with a fixed R value of 16 are shown in Fig. 1. All curves, whose half-wave potentials range from +0.01 to −0.07 V *vs.* S.C.E., are well-defined and reversible. In the pH region between 2.7 and 3.2, where PGA alone is known to be in a helical form, the current-potential curve is almost the same as that of a Cu(II) solution. As the pH of the Cu(II)–PGA solution is increased, the diffusion current decreases steadily. However, when the pH becomes higher than 4.5, the diffusion current approaches a finite value as can be seen from the figure. This polarographic behavior indicates that the formation of Cu(II)–PGA complex depends on pH.

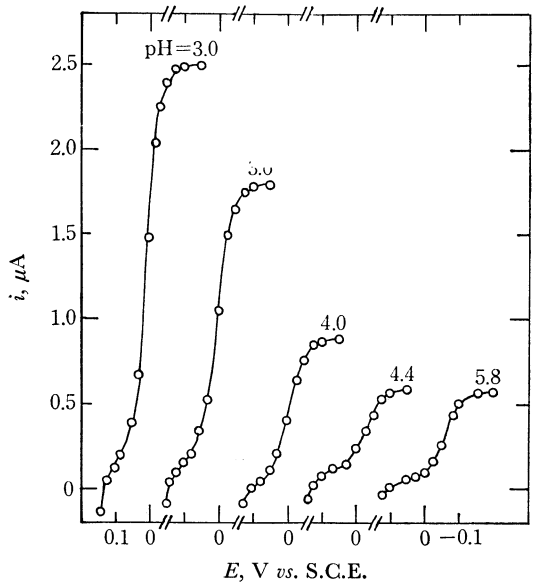

Fig. 1. The pH dependence of current-potential curves of Cu(II)–PGA at $R=16$. R=[PGA]/[Cu(II)]

The Relation between Diffusion Current and pH at Various Mixing Ratios. In Fig. 2, the diffusion currents are plotted against pH at various values of R. Each curve, which shifts toward high pH with decreasing R, shows a sharp decrease in $(i_d)_p$ that is confined in

16) K. Yamaoka, *Biopolymers*, **2**, 219 (1964).

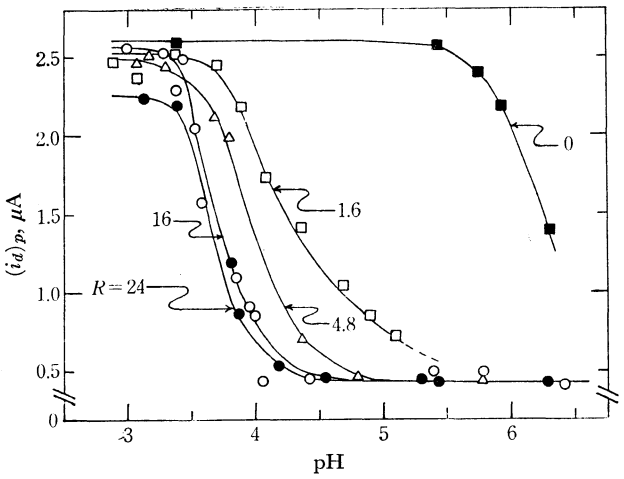

Fig. 2. The relation between diffusion current and pH for Cu(II)–PGA at various values of R as a parameter.[17]

a relatively narrow range of pH. No such change was observed for the Cu(II) aquocomplex solutions ($R=0$) in the same pH range between 3.0 and 5.4; however, the diffusion current gradually decreases as the pH value becomes higher than 5.4. This appears to be due to the formation of insoluble cupric hydroxide.[9] It should be noted here that the shape of the curves in Fig. 2 resembles very closely the pH-induced helix-coil transition of aqueous PGA and further that the shift of the curves as affected by the change in R is very similar to the trend induced by addition of various monovalent neutral salts.[17] This suggests that the Cu(II) ions coordinated with PGA ligands play an important role in the conformational transition of PGA. These conformational transition curves for R of 24, 16, and 4.8 are all similar in shape and approach a finite value which is independent of pH higher than 5.0. The measurements for R higher than 24 were not carried out, since the $(i_d)_p$–pH plot at $R=24$ could be almost superimposed upon that of $R=16$. On the other hand, as R decreases, not only the inflection point of each curve displaces for the higher pH, but also the slope becomes much more gradual (compare $R=24$ with 1.6). The polarographic measurement for $R=1.6$ was limited only up to pH around 5 due to the appearance of coagulated complex.

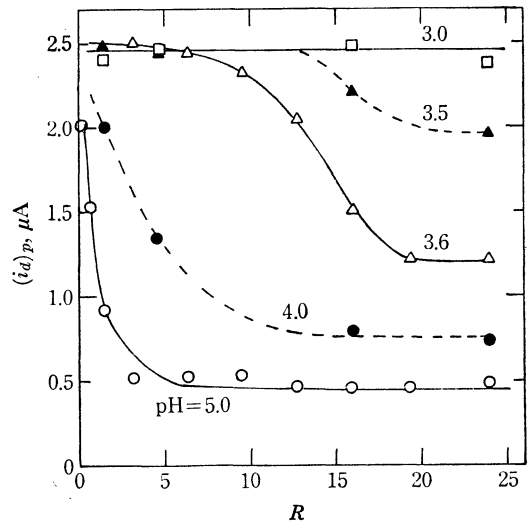

Fig. 3. The relation between diffusion current and mixing ratio at various pH's. Filled circles and triangles (dotted curves) are points read from the curves shown in Fig. 2.

17) Triton X-100 (0.01%) was used for the $R=0$ solution.
18) A. L. Jacobson, *Biopolymers*, **2**, 237 (1964).

The Relation between Diffusion Current and Mixing Ratio at Various pH's. In Fig. 3, the plots of $(i_d)_p$ *versus* R are shown at some representative pH's. Each of these curves shows its own characteristics. At pH 3.0, where the curves in Fig. 2 reach an upper plateau and apparently the Cu(II)–PGA complexes have the same gross conformation, presumably a rigid helix, the $(i_d)_p$–R curve is almost linear and independent of the values of R examined. At pH 3.6, where the complex may be a mixture of molecules in helical and in random-coil conformation or may possess the helical and random-coil segments in a single molecule, the $(i_d)_p$–R plot decreases gradually but steadily in the R region between 7 and 20, beyond which the curve levels off. Now, in the curve at pH 5.0, where the complex appears to be in a completely random-coil form as judged from $(i_d)_p$–pH curves in Fig. 2, the $(i_d)_p$ values decrease very sharply even at smaller values of R (between 0 and 3); beyond this, however, this curve approaches another plateau. It should be noted that this plateau is much lower than the one at pH 3.6 (see Discussion).

The Relation between Half-Wave Potential and Mixing Ratio at Two Extreme pH's. In Fig. 4, the effect of R on the half-wave potential is shown at the same two pH's as those in Fig. 3. The variation of $E_{1/2}$ with R is obvious at pH 5.0, but no such change is observed at pH 3.0 and 3.6 (not shown).

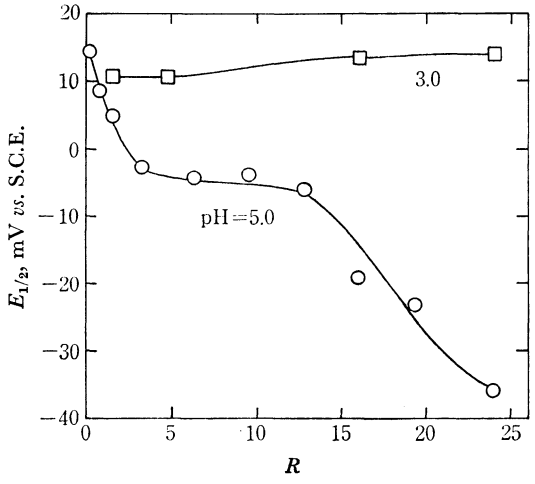

Fig. 4. The relation between the half-wave potential $E_{1/2}$ and the mixing ratio at pH's 3.0 and 5.0

The Effect of Temperature upon Diffusion Current at Two Extreme pH's. In order to obtain the activation energy of diffusion, Q_D, for the complexes in helical (pH 2.7) and random-coil (pH 5.0) conformations, diffusion currents were measured at four temperatures, 10, 20, 30, and 40°C. In Fig. 5, the plots of $\log[(i_d)_p/(m^{2/3}t^{1/6})]$ *versus* $1/T$ are shown at those pH's and at $R=16$. The slopes of the straight lines was found to be −470 and −760 (in degrees) at pH's of 2.7 (curve

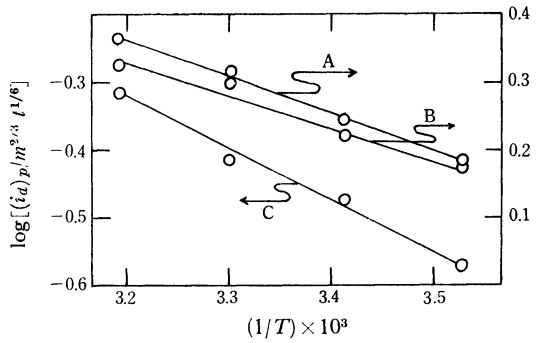

Fig. 5. The effect of temperature upon diffusion current.
Curve A: Cu(II) aquocomplex at pH 3.0;
Curve B: Cu(II)–PGA complex at pH 2.7;
Curve C: Cu(II)–PGA complex at pH 5.0.
Arrows indicate the ordinate to be referred to.

B) and 5.0 (curve C), respectively. A value of −550 was obtained for Cu(II) aquocomplex at pH 3.0 (curve A) for comparison. From these values the activation energy of diffusion can be calculated with the following equation derived by Vlček.[19]

$$\log \frac{(i_d)_p}{m^{2/3}t^{1/6}} = \log (0.627 \cdot n \cdot C \cdot \boldsymbol{F} \cdot D_0^{1/2}) - \frac{\frac{1}{2}Q_D}{2.3RT} \quad (1)$$

where C is the concentration of Cu(II) ions; $\boldsymbol{F}$, the Faraday constant; D_0, a constant. The values of activation energy for Cu(II)–PGA complexes at two pH's and for Cu(II) aquocomplex at pH 3.0 are listed in Table 1.

Table 1. The activation energy of diffusion, Q_D

Sample	pH	R	Q_D (kcal)
Cu(II)–PGA (helical)	2.7	16	4.3
Cu(II)–PGA (random)	5.0	16	6.9
Cu(II) (aquo)	3.0	0	5.0

Discussion

The Role of the Cu(II) Ion in the Conformational Transition of PGA. The observed fact that the diffusion current of Cu(II)–PGA complexes at various values of R abruptly increases with decrease in pH, as shown in Fig. 2, should correspond to the pH-induced helix-coil transition of PGA. This conclusion on the behavior of $(i_d)_p$–pH curves of Cu(II)–PGA complexes is strongly supported by an independent experiment, under the conditions similar to those in this work, of the complex between Cu(II) and monomeric sodium glutamate which shows that the diffusion current does not change over the pH range between 3 and 7.[20] Moreover, the almost parallel shifts of the $(i_d)_p$–pH curves toward higher pH with decrease in R in Fig. 2 might be interpreted that the formation of such Cu(II)-rich complex stabilizes the secondary or helical structure of the polymer.[7]

It is interesting to compare the present results with the report by Takesada *et al.*[7] who noted that the pH-induced helix-coil transition curves in the presence of Cu(II) ions, studied by the method of optical rotatory dispersion, shifted in a similar manner. They observed that, although up to R larger than 20 the transition behavior of Cu(II)–PGA solutions was very close to that of a PGA solution, the slope of the pH-induced transition curves for values of R smaller than 6.6 became more gradual. They attributed their observation to the retention of a helical structure even at a relatively high pH region in the presence of a sufficient number of Cu(II) ions. The transition points in their work range from pH 6.3 to 7.1 (polymer concentration, 0.1 mg/m*l*), whereas the polarographic transition points in our study defined as the midpoint of the increment of diffusion currents, were confined between pH 3.7 and 4.5 (polymer concentration, 1.5 mg/m*l*). This discrepancy is beyond the experimental error and is unable to be attributed to the difference in concentration of polymer or neutral salt.[7] Whether or not it results from the difference in experimental methods employed remains to be resolved.

It is true, in fact, that the polarographic transition point is dependent on the concentrations of the Cu(II) ion and the ligand. As shown in the earlier paper,[6] the point was located at pH 4.2 for the Cu(II)–PGA complex in which the concentrations of the Cu(II) ion, PGA and NaCl were all kept at one-fifth of those employed in this work (in both cases R remained at 16). This is compared in Fig. 6 where the difference of one-half pH unit for two transition points is clearly noted. It should be stated here that the same trend was reported by Takesada *et al.*[7]

The Estimation of the Helical Content of the Complex at Various Values of R. Based on the plots in Fig. 3, it is possible to estimate the helical content of the Cu(II)–PGA complexes in a certain range of R at a

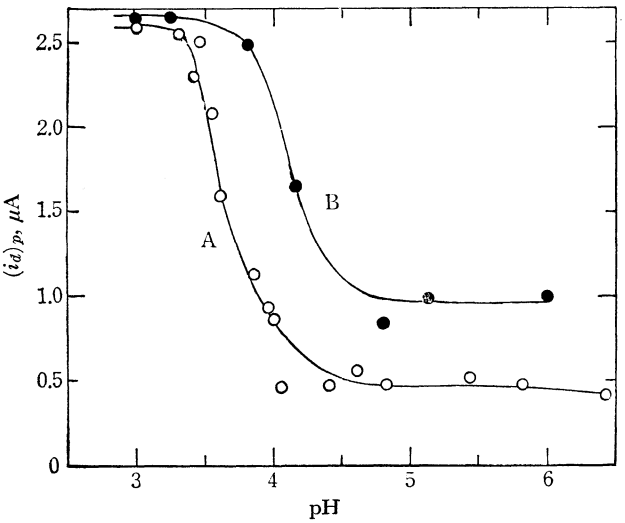

Fig. 6. The diffusion current *vs.* pH curves for two Cu(II)-GPA samples having the same R value of 16 but different concentrations for individual components.
Curve A: $[Cu(II)]=5\times10^{-4}$M, $[PGA]=8\times10^{-3}$M, $[NaCl]=7.5\times10^{-3}$M.
Curve B: $[Cu(II)]=10^{-4}$M, $[PGA]=1.6\times10^{-3}$M, $[NaCl]=1.5\times10^{-3}$M.
The scale of $(i_d)_p$ for curve B is expanded by a factor of 5. These data are taken from Ref. 6.

19) A. A. Vlček, *Collection Czechoslov. Chem. Commun.*, **24**, 3538 (1959).
20) To be published.

given pH. Here, we make the following assumptions. (1) The value of the diffusion current at pH 3.0, which is designated as $(i_d)_p^h$, represents the diffusion current of a Cu(II)–PGA helix. (2) Similarly the diffusion current of the complex at pH 5.0 in the range of R larger than 5 is due to random-coil PGA molecules which completely coordinate to all Cu(II) ions present in solution; this value is designated as $(i_d)_p^r$. The helical fraction, f_h, at a given pH and R, at which the observed diffusion current is $(i_d)_p$, is then given by the following expression

$$f_h = \frac{(i_d)_p - (i_d)_p{}^r}{(i_d)_p{}^h - (i_d)_p{}^r} \tag{2}$$

For a Cu(II)–PGA complex at pH 3.6, f_h is calculated to be about 0.37 in the range of R larger than 20 where $(i_d)_p$ is constant. Similarly an estimate is made for the complex at pH 4.0, in which f_h is 0.11 in the range of R larger than approximately 10.

Now, the question arises on what extent of helical fraction a complex possesses at a given pH and R where the diffusion current is continuously changing (*i.e.*, in the range of R between 5 and 18 for pH 3.6 and between 0 and nearly 10 for pH 4.0, *cf.* Fig. 3). There are two interpretations possible from the present data only. At first, the helical fraction of the complex at a fixed pH of 3.6 steadily increases as R becomes smaller, *i.e.*, the more Cu(II) ions are added, the more stable does the helix become. This is the view promoted by Takesada *et al.*[7] based on the results of an ORD study; however, this interpretation is not unique. An alternative is that the steady increase in $(i_d)_p$ for R smaller than 18 is in actuality the reflection of a chemical process in which the number of the bound Cu(II) ions remains constant at a certain level regardless of apparent values of R; in other words, the increase in $(i_d)_p$ is mostly due to free Cu(II) ions. If this is true, the notion that the presence of the Cu(II) ion in PGA facilitates or enhances the helical formation must critically be reexamined.

The Estimation of the Molecular Size of the Complex from Diffusion Currents. From analysis of diffusion currents, we are able to estimate the molecular size of Cu(II)–PGA complex in a random-coil conformation and to obtain some insight into the mechanism of the interaction between the Cu(II) ion and PGA in a helical conformation. Assuming that the Cu(II)–PGA complex at pH 5.0 is an impenetrable, rigid sphere and that the Stokes-Einstein equation (Eq. (3)) can be applied to the polarographic diffusion process prior to electrode reduction, the radius of such a sphere, r, can be estimated by

$$r = \frac{RT}{N}\frac{1}{6\pi\eta D} \tag{3}$$

where R is gas constant; N, the Avogadro number; T, the absolute temperature (=298°K); η, the viscosity of water ($=0.89\times10^{-3}$ g/cm·sec at 25°C). The diffusion coefficient, D, is calculated with the aid of Ilkovič's equation;

$$D = \left[\frac{(i_d)_p}{605\cdot n\cdot C\cdot m^{2/3}\cdot t^{1/6}}\right]^2 \tag{4}$$

where the following numerical values are adopted:

Table 2. Diffusion coefficients and other pertinent data

	Concentration (mM)	pH	Mixing ratio	$(i_d)_p$ μA	$D\times10^7$ cm²/sec
Cu(II)–PGA	0.5	5.0	16	0.37	7.36
Cu(II)–PGA	0.5	3.0	16	2.50	77.3
Cu(II)–PGA	0.1	5.0	16	0.20	9.00
Cu(II)–PGA	0.1	3.0	16	0.50	68.0
Cu(II)	0.5	3.0	0	2.60	78.0
Cu(II)	0.1	3.0	0	0.53	63.0
Cu(II)	—	—	—	—	72.0[a]
In(III)	1	—	—	—	96[b]

a) I. M. Kolthoff, and J. J. Lingane, "Polarography", Vol. 1, Interscience Publishers, New York, N. Y., (1952) p. 52.
b) S. Inoue, and H. Imai, This Bulletin, **33**, 149 (1960).

$(i_d)_p=0.37$ μA, $C=0.5$ mM, $n=2$, $m=0.989$ mg/sec, $t=8.14$ sec at pH 5.0. In Table 2 the diffusion coefficients of the complexes and other pertinent data are tabulated. As can be seen from Table 2, the diffusion coefficients calculated from our data are in good agreement with those of previous workers. The radius of the assumed sphere of the Cu(II)–PGA complex at pH 5.0 is then calculated to be 33 Å, the value of which yields a molecular volume of 1.5×10^5 Å³. This value is quite consistent with a calculated volume for PGA (details will be published in due course).

The Estimation of the Coordination Number of Cu(II)–PGA Complexes in a Random Conformation. If an electrode reaction proceeds reversibly[21] by the reduction of a complexed Cu(II) ion to a metallic state at the dropping mercury electrode, then the following expression can be applied to obtain the coordination number, j, of such a complex at a given temperature.[22]

$$\frac{d(E_{1/2})}{d(\log C_x)} = -j\frac{0.0591}{n} \tag{5}$$

Thus, from the slope of the $E_{1/2}$-log C_x curve, a value of j can be evaluated. To derive this expression the following assumptions are made:[21] (1) the diffusion current constants of both metallic ion and complex are independent of the respective concentrations, (2) a term containing the activity coefficients of chemical species in solution may be dropped out, and (3) C_x should be the concentration of the free ligands which, however, can be approximated as the total concentration of ligands.

In Fig. 4, the $E_{1/2}$ of Cu(II)–PGA complex was shown to vary in a distinctive manner with the value of R at pH 5.0 where the complex is in a random form, in contrast with the $(i_d)_p$ which, as shown in Fig. 3, remained constant for R larger than 5.0. The $E_{1/2}$ values were replotted in Fig. 7 against the logarithm of the total concentration of PGA in solution, C_x, which is equal to R times the concentration of the Cu(II) ion. Two linear portions cross at log $C_x=-2.2$. From the slope of the straight line A, a j value of 3.4 is yielded. Simi-

21) This assumption was verified by determining the slope of a plot of $\log[(i/(i_d)_p-i)]$ versus electrode potentials. A value of two was assumed for n.
22) D. R. Crow, "Polarography of Metal Complexes", Academic Press, London, 1969, p. 62.

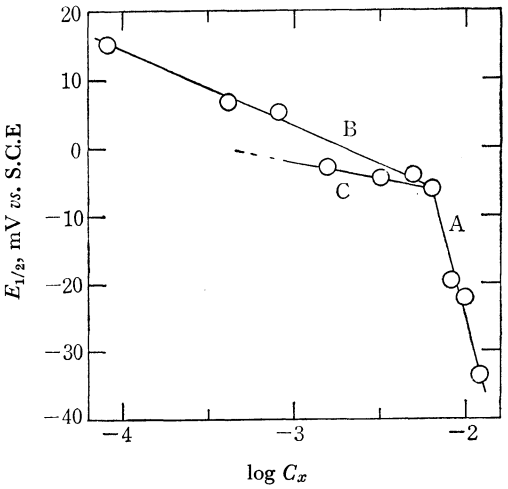

Fig. 7. The Relation between half-wave potential and logarithm of the total concentration of PGA at pH=5.0 and R=16. $C_x=R\cdot$[Cu(II)]

larly, the lines B and C result in j values of 0.34 and 0.17, respectively. It should be noted that the portions A and C correspond to the range of R (from 3 to 24) where $(i_d)_p$ remains unchanged (see Fig. 3). From these j values it is clear that the number of binding sites on each PGA residue changes with the value of R or the concentration of PGA. The coordination number of 3.4 (which reduces to 2.9 when the concentration of free ligands is taken for C_x) indicates that approximately three peptide residues coordinate to each Cu(II) ion as an average. Since each residue possesses three capable sites—amide nitrogen and oxygen atoms and a carboxyl side chain, nine binding sites at maximum are allowable to participate in complex formation. The exact number or stereochemistry, however, is undeterminable.

Assumed that the sharp increase in $(i_d)_p$ at pH 5.0 ($R<3$) shown in Fig. 3 is entirely due to the free Cu(II) ion released from the complex and, consequently, the line B has no meaning, the j value of 0.17 from line C should be interpreted as due to that each PGA residue apparently coordinates to about five Cu(II) ions. This result excludes the possibility that the same type of chelation as occurred in portion A also exists. Therefore, a most plausible mode of binding in portion C is an electrical or ionic pairing with PGA residue. However, if the entire portion of B is meaningful and the whole change in the $(i_d)_p$–R curve at pH 5.0 is assumed to be caused by the electrostatic interaction as described in Results, the j value of 0.34 means that each residue coordinates to three Cu(II) ions. The differentiation of these two ideas awaits a further study. In any case the data in Fig. 7 clearly show that the binding mode of Cu(II) ion to PGA residue changes with the mixing ratio even if the gross conformation of the complex is random-coil.

The Activation Energy of Cu(II)–PGA Complexes in Helical and Coiled Conformations. As can be seen in Figs. 3 and 4, the binding mode of Cu(II)–PGA complex in helical form should differ from that in coiled form. The data in these figures should reflect the difference of the diffusion of these complexes toward the electrode prior to electrode reduction. The difference between the value of Q_D of the Cu(II)–PGA complex at pH 2.7 and that of the complex at pH 5.0 listed in Table 1 clearly indicates that the mechanism of diffusion is affected by the secondary structure of Cu(II)–PGA complex. The finding that the Q_D value of the complex in helical conformation is almost the same as that of the free Cu(II) aquocomplex suggests that the interaction between Cu(II) ion and helical PGA molecule is weak, if any; hence, the loose interaction is likely to be ionic in nature. As the complex diffuses toward electrode, the loosely bound Cu(II) ions may be unsheathed or "zipped-off" to reduce at the electrode surface. The activation energy of diffusion for Cu(II)–PGA complex at pH 5.0 is 60% greater than that at pH 2.7. This means that Cu(II) ions are tightly or coordinately bound with PGA in the coiled region, and that the complex diffuses to the surface as a whole. Or alternatively, the amount of energy corresponding to break the bonds between Cu(II) ion and PGA is required to make unbound free aquo-ions diffuse.

Finally, from the present and earlier studies,[6] it is concluded that the relationship between the polarographic diffusion current and pH is clearly established and definitely reflects the pH-induced helix-coil transition of Cu(II)–PGA complex. These polarographic studies enable to provide rich information on characteristics of the complex in aqueous solutions such as molecular size, helical fraction, mode of coordination, and activation energy of diffusion.

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