February, 1986] © *1986 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.,* *59,* *587—591 (1986)* 587

Effects of Bivalent Metal Cations on the Conformation and Aggregation of Poly(L-glutamic acid)

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(Received September 25, 1985)

In the solution of fully neutralized poly(L-glutamic acid) in the absence of supporting electrolytes, the α-helix was induced by the addition of five transition metal chlorides in the following order: $CuCl_2 \gtrsim CdCl_2 > ZnCl_2 > NiCl_2 > CoCl_2$. The induction did not occur by the addition of $BaCl_2$, $CaCl_2$, or $MgCl_2$. The induction by transition metal cations was inhibited in the presence of a supporting electrolyte (NaCl). Distortion of circular dichroism (CD) spectra occurred as the induction of the α-helix proceeded, which was shown to be recognizable when apparent aggregation number m exceeded about 100. Conformation of the polypeptide in these aggregates was expected to be the α-helix based on the conformation in the precipitates obtained at these mixing ratios which gave distorted CD spectra, which was determined by infrared absorption spectra.

Induction of the secondary structures of fully neutralized anionic polypeptides was studied on poly(L-glutamic acid)(PGA)[1] and poly (*S*-carboxymethyl-L-cysteine)(poly[Cys(CH_2COOH)])[2] by the addition of cationic surfactants of different head groups but of a common hydrocarbon tail. Different behaviors were observed between these two polypeptides. The induction of the α-helix of PGA occurred for three surfactants with small differences among their inducing powers,[1] while the induction of the β-structure of poly[Cys(CH_2COOH)] depended on the kind of head groups.[2] Furthermore, in the presence of 0.1 M NaCl, the induction of the α-helix of PGA was completely inhibited,[1] while that of the β-structure of poly[Cys(CH_2COOH)] was scarcely perturbed.[2]

It is interesting to examine whether such a definite difference exists between the inductions of the α-helix and the β-structure caused by the addition of bivalent metal cations. Effects of bivalent metal cations on the conformation of poly[Cys(CH_2COOH)][3] and its side chain homolog[4] were already examined. Interactions of bivalent metal cations with PGA has been extensively studied,[5–15] including the induction of the α-helix by Cu^{2+} and Cd^{2+} ions. Nevertheless, available data for the present purpose of comparison are not sufficient for PGA. Therefore, previous studies[3,4] are extended to the induction of the α-helix of PGA in the present study.

Another purpose of the present study is clarifying the origin of the distortion of the circular dichroism (CD) spectra of PGA. The distortion of CD has been attributed to the aggregation of α-helices,[16,17] or to the β-structure[15] without providing evidence for both aggregation and conformation. In the present study both the aggregation number and the conformation of the polypeptide in the aggregates are examined.

Experimental

Weight average molecular weight and the degree of polymerization of the used PGA sample were 6.3×10^4 and 480, respectively, as determined from light scattering. Reagent grade $CuCl_2 \cdot 2H_2O$, $CdCl_2 \cdot (1/2)H_2O$, $NiCl_2 \cdot 6H_2O$, and $BaCl_2 \cdot 2H_2O$ were purchased from Nakarai Chemicals Ltd. (Kyoto). Reagent grade $ZnCl_2$ was from Wako Pure Chemicals or prepared by the reaction of Zn with HCl. $CaCl_2 \cdot 4H_2O$ (suprapur) and $CoCl_2 \cdot 6H_2O$ were from Merck. $MgCl_2 \cdot 6H_2O$ was from Baker.

Circular dichroism (CD) was measured with a Jasco J-40 A circular dichrograph using a cell of 1 mm light path at 25±0.1 °C. Light scattering was mesured on a Chromatix KMX-6 low-angle light-scattering photometer at ambient temperature (24±2 °C). A 6—7° annulus was chosen to observe the scattered light which collected the light of about 4—5° scattering angle for aqueous solutions. A 0.2 mm field stop was selected which defined the scattering volume. Millipore membrane filters of 0.1, 0.22, and 0.30 µm pore size were used to filter the solutions containing aggregates of aggregation number smaller than 12, about 53 to 170, and greater than 250, respectively. Measurements of pH were carried out with an Iwaki M-225 pH meter. Infrared absorption spectra of precipitates in KBr pellets were taken with a Jasco IRA-2 spectrophotometer at 24±2 °C. Precipitates of PGA-metal ion complexes were collected by centrifugation(ca. 1500 g), dried in a desicator over P_2O_5 for one or 2 d, and then mixed with dry KBr powder. The mixture was ground finely and kept in a desicator over P_2O_5 for several days. The mixture was pressed (6—7 t/cm^2) to a pellet for 3 min in vacuo. The pH of the solutions prior to the addition of a metal chloride was adjusted to be 6.7±0.2 by the addition of NaOH, if necessary. Then, various amounts of a metal chloride were added and the mixtures were incubated for 12—24 h at 24±2 °C before the measurements. Concentrations of the polymer C_P and metals C_M are expressed in residue molarity and molarity M ($mol\,dm^{-3}$), respectively.

Results

Induction of the α-Helix. In Fig. 1 are shown the CD spectra of the solutions containing 1 mM PGA and various amounts of $NiCl_2$. On addition of $NiCl_2$, the α-helix was induced as indicated by a change of CD spectra. The helical content was about 75% at the mixing ratio C_M/C_P of 2.0. Further addition of $NiCl_2$ did not enhance the α-helix formation but distorted the CD spectra (curve f) and eventually led to the precipitation. No isodichroic point is seen in Fig. 1. The same behavior was observed on the addition of $CoCl_2$. On the contrary, an isodichroic point was found

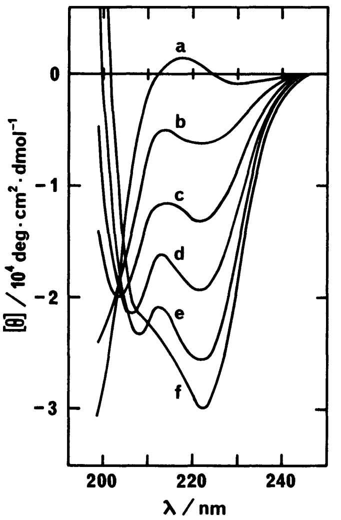

Fig. 1. CD spectra of PGA (C_p=1 mM) in the presence of various amounts of $NiCl_2$. Mixing ratio C_M/C_p. (a): 0, (b): 0.17, (c): 0.27, (d): 0.50, (e): 1.0, and (f): 6.0.

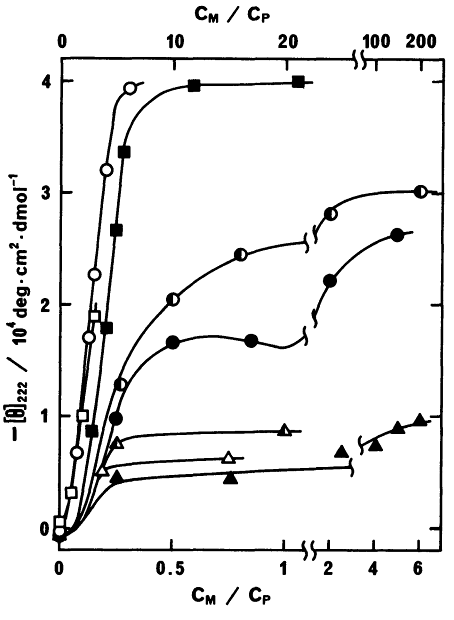

Fig. 2. Dependence of $[\theta]_{222}$ on the mixing ratio C_M/C_p. (○): $CuCl_2$, (□): $CdCl_2$, (■): $ZnCl_2$, (◐): $NiCl_2$, (●): $CoCl_2$, (△): $BaCl_2$, (▲): $CaCl_2$, and (▲): $MgCl_2$. Bottom and top scales of abscissae refer to transition metals and alkaline earth metals, respectively.

around 203—204 nm in the α-helix induction by $CuCl_2$ or $CdCl_2$. This behavior is consistent with the reported one.[11] Change of the CD spectra on the addition of $ZnCl_2$ was an intermediate one between these two types. Since a distortion of the CD spectra at high mixing ratios occurred around 210 nm, the residue ellipticity at 222 nm ($[\theta]_{222}$) was used as a measure of the helical content.

In Fig. 2, the residue ellipticities at 222 nm are plotted against the mixing ratio C_M/C_P for eight bivalent metal chlorides. Induction of the α-helix occurred completely on the addition of $CuCl_2$ or $ZnCl_2$, while precipitation occurred before the induction was completed in the case of $CdCl_2$, $NiCl_2$, or $CoCl_2$. Contrary to these five transition metals, $BaCl_2$, $CaCl_2$, or $MgCl_2$ did not induce the α-helix. In the range of C_M/C_P below about 5, $[\theta]_{222}$ varied from nealy zero (or small positive) to −3000—−8000 for these three alkaline earth metals. No further change occurred, however, in the range of C_M/C_P greater than about 5. Consequently, the change of CD spectra can be ascribed to a kind of the reported effect of ionic strength[18] rather than to a partial conversion to the α-helix.

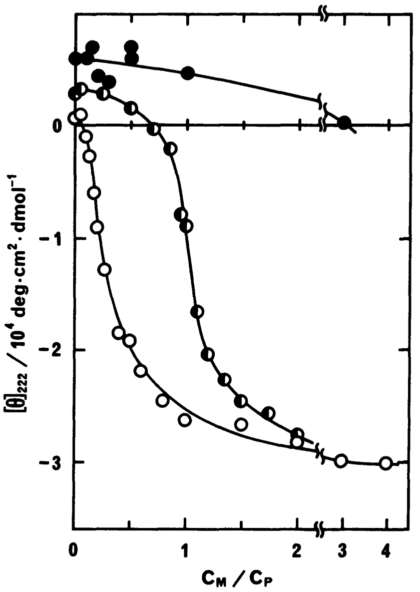

Fig. 3. Effects of NaCl on the induction of the α-helix by the addition of $NiCl_2$. C_p=1.0 mM. NaCl concentration (M). (○): 0, (◐): 0.010, and (●): 0.10.

Precipitation of the polypeptide occurred in the range of C_M/C_P greater than about 30 in the case of $BaCl_2$ or $CaCl_2$, while precipitation did not occur at C_M/C_P=200 in the case of $MgCl_2$. The change of $[\theta]_{222}$ occurred in one step for every bivalent cation, although apparently two-step changes are seen in Figure 2 for Ni^{2+}, Co^{2+}, and Mg^{2+} simply because abscissa scales are changed.

Effect of Supporting Electrolyte. The effect of $NiCl_2$ on the conformation of PGA was also examined in 0.01 M and 0.1 M NaCl solutions. The result is shown in Fig. 3, together with the result in the absence of NaCl for the sake of comparison. In 0.01 M NaCl, the induction of the α-helix was hampered when C_M/C_P was smaller than about unity but became comparable with that in the absence of NaCl in the range of C_M/C_P between 1.5 and 2. Precipitation occurred when C_M/C_P was greater than about 2. In 0.1 M NaCl, the induction did not occur up to C_M/C_P=3 and precipitation occurred on further addition of $NiCl_2$. The inductive effect of $NiCl_2$ was thus largely suppressed in the

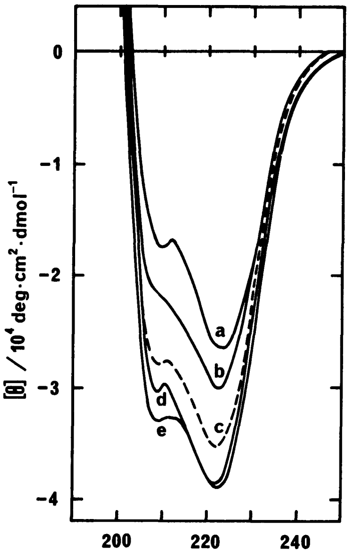

Fig. 4. CD spectra at the mixing ratios slightly smaller than those caused precipitation. Mixing ratios (C_M/C_P). (a): $CoCl_2$ 5.0, (b): $NiCl_2$ 6.0, (c): $CdCl_2$ 0.275, (d): $ZnCl_2$ 0.82, and (e): $CuCl_2$ 0.30. The CD curve (c) was obtained on turbid solutions.

presence of 0.1 M NaCl. This inhibitory action of ionic strength is similar to that found on the interaction of PGA with surfactants,[1] or other polyion-bivalent metal interactions.[19,20] This behavior is in contrary to the effect of ionic strength on the induction of β-structure of poly(*S*-carboxymethyl-L-cysteine) by bivalent metal cations.[3] Also, it is reported that interaction of $Cu(NO_3)_2$ with poly(methacrylic acid) was not affected by the presence of 0.1 M $NaNO_3$.[21]

Distortion of the CD Spectra. When a transition metal chloride was added to such an amount more than required to the maximum induction for that species, the CD spectra began to be distorted in a way that $[\theta]_{208}$ decreased in magnitude. The CD spectra obtained at the mixing ratio near but slightly smaller than that caused precipitation are shown in Fig. 4 for five transition metal chlorides. Distortion occurred only slightly in the case of $CuCl_2$. In the case of $CdCl_2$, the spectra were obtained on a turbid solution and hence represented by a dashed curve c. These spectra shown in Fig. 4 might be associated with the presence of the β-structure.

The distortion of the CD spectra can be clearly discriminated when the correlation between $[\theta]_{208}$ and $[\theta]_{222}$ is examined (Fig. 5) as proposed previously.[1] A straight line in Fig. 5 represents the induction of the α-helix, probably including the effect of ionic strength on the CD spectra. The data of $CdCl_2$ located in the range $-[\theta]_{222}>2\times10^4$ were taken on turbid solutions. Deviation from the straight line occurs at different helical contents for different species; the following order of the helical content is seen, $CoCl_2<NiCl_2<CdCl_2<ZnCl_2$. The deviation is not clear for $CuCl_2$.

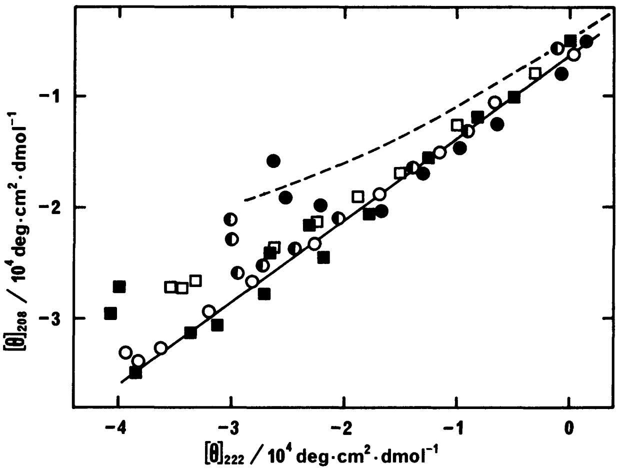

Fig. 5. Correlation between $[\theta]_{222}$ and $[\theta]_{208}$ in the presence of various amounts of a given metal chloride. The same symbols are used as in Figure 2 to represent different metal species. A dashed curve represents the result of the addition of $NiCl_2$ in 0.01 M NaCl.

A dashed line in Fig. 5 represents the result on the addition of $NiCl_2$ in 0.01 M NaCl. Distortion of the CD spectra also occurred in 0.01 M NaCl as shown by a nonzero curvature of the dashed line.

Aggregation of PGA in 0.01 M NaCl. Light scattering was measured on the 0.01 M NaCl solutions containing 1 mM PGA and various amounts of $NiCl_2$. Apparent aggregation number *m* was evaluated by Eq. 1.

$$m = \Delta R_\theta(C_M)/\Delta R_\theta(C_M=0) \quad (1)$$

In Eq. 1, ΔR_θ represents a difference of light intensities between a solution and a solvent at a scattering angle θ, which is very close to zero in the present study. Underlying assumptions leading to Eq. 1 are: (1) refractive index increment does not vary appreciably on addition of $NiCl_2$ and (2) the second virial coefficient can be ignored for any aggregates. (In the absence of $NiCl_2$, the contribution from the second virial coefficient did not exceed 5%) In spite of these approximations, the apparent aggregation number *m* is expected to be a useful measure for the extent of aggregation. When the dependence of *m* on the mixing ratio given in Fig. 6 is compared with that of $[\theta]_{222}$ given in Fig. 3, it is seen that aggregation occurs at small mixing ratios corresponding to low helical contents. For example, at $C_M/C_P=1.0$, $-[\theta]_{222}$ is smaller than 10^4, yet aggregation number already reaches 167. If every two carboxylate groups are assumed to bind to a Ni^{2+} ion, then *m* should be corrected to 136 instead of 167. This provides a rough estimate of the consequence of assumption (1).

As a measure of the extent of distortion of CD spectra, the slope of the dashed curve given in Fig. 5 was

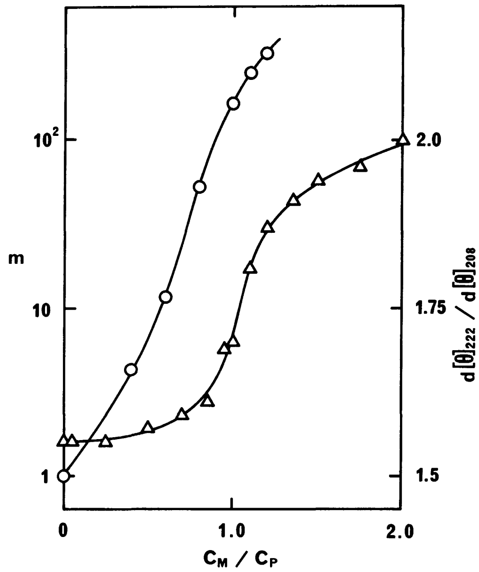

Fig. 6. Apparent aggregation number m and a measure of the distortion of the CD spectra, $d[\theta]_{222}/d[\theta]_{208}$, as functions of the mixing ratio (C_M/C_P) of $NiCl_2$ in 0.01 M NaCl. (○) m and (△) $d[\theta]_{222}/d[\theta]_{208}$ refer to the left and right ordinate scales, respectively.

tentatively employed which is represented by Eq. 2.

$$[\theta]_{208}/10^4 = 0.473([\theta]_{222}/10^4)^2 + 0.631([\theta]_{222}/10^4) - 0.050 \quad (2)$$

Equation 2 was obtained from the exeprimental points by the least square method. The slope, $d[\theta]_{222}/d[\theta]_{208}$ was evaluated by differentiating Eq. 2. The slope is 1.55—1.60 in the range well approximated by a straight line. When $C_M/C_P>1.0$, the slope increases sharply to 1.80 or greater. Consequently, it is concluded that the distortion of the CD spectra becomes first recognizable when m is larger than about 100.

Infrared (IR) Absorption Spectra of Precipitates. At the mixing ratio where the distorted CD spectra shown in Fig. 4 were obtained, precipitation occurred when the polypeptide concentration exceeded 1 mM. Consequently, conformation of the polypeptide in the precipitates rather than in solution was examined by infrared absorption spectra. The IR spectra of the precipitates are shown in Fig. 7 obtained from the solution of C_P=10 mM by the addition of $CuCl_2$, $NiCl_2$, or $CoCl_2$. Amide I, II, and V bands were found at 1650—1655 cm^{-1}, 1550 cm^{-1}, and 630 cm^{-1}, respectively, for these three complexes. These band positions indicated that the conformation of PGA was the α-helix.[22–24]

A band around 1620 cm^{-1} was observed in the complex with $CuCl_2$, which can be assigned to antisymmetric stretching of side chain carboxylato group covalently bound to a copper atom,[25,26] rather than to the amide I band in the β-structure. There is no room to expect the presence of the β-structure in the case of $CuCl_2$, since distortion of the CD spectra did not occur appreciably. Corresponding bands could not be resolved in the case of $NiCl_2$ or $CoCl_2$.

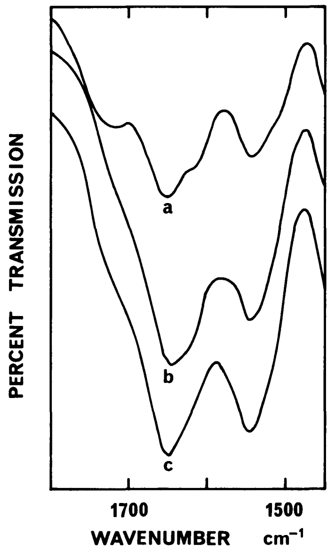

Fig. 7 Infrared absorption spectra of the precipitates in KBr disks consisting of PGA and (a): $CuCl_2$, (b): $NiCl_2$, or (c): $CoCl_2$.

The β-structure is one of stable conformations of PGA in the solid state.[27–31] The presence of the β-structure of PGA in solution has not been confirmed until now. Furthermore, dissolution of the β-precipitates of PGA was reported to be very difficult.[30] Based on all these findings, it is quite unlikely that the conformation of the polypeptide changed from the β-structure to the α-helix during the precipitation in the present study. We can, therefore, conclude that the conformation of PGA in soluble aggregates is the α-helix.

Discussion

Induction of the α-Helix as Compared with That of the β-Structure. Induction of the α-helix of PGA in the media of no added salt occurred in the present study by the addition of transition metal chlorides in the order of $Cu^{2+} \gtrsim Cd^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+}$. On the other hand, alkaline earth metals ($BaCl_2$, $CaCl_2$, $MgCl_2$) did not induce the α-helix. This result is in good agreement with that found on the induction of the β-structure of poly[Cys(CH_2COOH)].[3] Furthermore, the order of the inducing power of transition metal cations is also in good agreement with the interaction of poly(methacrylic acid) with these metal cations.[21] Contrary to the induction by cationic surfactants, where clearly different behaviors were found between the inductions of the α-helix[1] and the β-structure,[2] the induction of the secondary structures by bivalent metal cations is similar for both the α-helix. and the β-structure.[3]

The interaction of transition metal cations with these two polypeptides, PGA and poly[Cys(CH_2COOH)], is much stronger than that of cationic surfactants. Difference of the stabilities between the α-helix and the β-structure escapes detection in the strong interaction with transition metal cations, while it is sensitively detected by the weak interaction with cationic surfactants.

However, clear difference between the interactions operating in the induction of the α-helix and the β-structure shows up when the effect of ionic strength is examined. The induction of the β-structure requires greater stabilization than that required for the α-helix induction. Hence the β-induction is scarcely affected by ionic strength of the environment, while the α-induction is effectively inhibited as the ionic strength increases.

Distortion of the CD Spectra. Distortion of CD spectra of the α-helix just as found in the present study has been often observed before.[1,16,17,32,33] Theoretical calculations show that the distortion occurs as a result of the aggregation of α-helices.[16,33,34] Comparison of the distorted CD spectra found in the present study with the calculated spectra[34] provides an estimate of the radius of assumed spherical aggregates to lie between 0.03 and 0.1 μm. On the other hand, the solution of $C_M/C_P=1.0$ could be filtered without trouble with a membrane of 0.22 μm pore size but not with a membrane with 0.1 μm pore size. Consequently, it is expected that the largest dimension of the aggregates in the solution exceeds 0.1 μm and that the aggregates have rather asymmetric shape but their optical properties are well approximated by spheres having a radius lying between 0.03 and 0.1 μm.

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