Induction of the β-Form of Poly(*S*-carboxymethyl-L-cysteine) and Poly[*S*-(2-carboxyethyl)-L-cysteine] by Chromium(III), Iron(III), and Lanthanum(III) Chlorides

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(Received October 7, 1983)

The β-form was induced by the addition of $CrCl_3$, $FeCl_3$, and $LaCl_3$ to the salt-free solutions of fully ionized poly(*S*-carboxymethyl-L-cysteine) (poly[Cys(CH_2COOH)]) and poly[*S*-(2-carboxyethyl)-L-cysteine] (poly[Cys(CH_2CH_2COOH)]). The induction occurred below the equivalent point in both polypeptides. The inducing powers of the three trivalent cations were rather similar. They were weaker than Cu^{2+} ions in the case of poly[Cys(CH_2COOH)], while they were comparable with Cd^{2+} ions in the case of poly[Cys(C_2H_4COOH)]. Cu^{2+} and Cd^{2+} ions were the most effective species among divalent cations in the induction of the β-form of poly[Cys(CH_2COOH)] and Poly[Cys(C_2H_4COOH)], respectively.

Recently, the effects of various divalent metal ions on the conformation of poly[Cys(CH_2COOH)]1) and poly[Cys(C_2H_4COOH)]2) were studied. Transition metal ions, such as Cu^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+}, effectively induced the β-form of these two polypeptides, while alkaline earth ions (Mg^{2+}, Ca^{2+}, and Ba^{2+}) were not effective inducers. Based on these results, it is likely that the β-form of these polypeptides is induced *in solution* only when their negative charges are annihilated by the redistribution of charges between the side chains and bound cations. In the present study, the effects of three kinds of trivalent metal chlorides, $CrCl_3$, $FeCl_3$, and $LaCl_3$, on the conformation of both polypeptides are examined in the fully ionized state.

Experimental

Poly[Cys(CH_2COOH)] (Lot No. KO125W; M_w=12 000, Dp=75) and poly[Cys(CH_2CH_2COOH)] (Lot No. H920WR; M_w=16 000, Dp=92) were the same samples as used in the previous studies.1,2) Here M_w and Dp represent the molecular weight and the degree of polymerization, respectively. Reagent grade $CrCl_3 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$, and $LaCl_3 \cdot 7H_2O$ were purchased from Nakarai Chemicals Ltd. (Kyoto) and used without further purification. Doubly distilled water was used to prepare the solutions.

Circular dichroism (CD) spectra were taken on a JASCO J-40 A circular dichrograph using a cell of 1 mm path length. Measurements of pH were carried out on a Hitachi-Horiba F-7 ss pH meter. These measurements were carried out at 25±2 °C. The polymer concentration C_p is expressed in the residue molarity, mol dm^{-3} (M), which is kept constant at 1.0×10^{-3} M in the present study. Metal concentration C_M is expressed in molarity (M), mol dm^{-3}. To compare the effects of trivalent ions with those of divalent ions, the metal concentrations are also expressed in normality (equiv dm^{-3}) and designated as C_s. Stock solutions of metal chlorides were used within half an hour after their preparation. After metal solutions were added to the polypeptide solutions, the mixtures were incubated for at least 24 h before the measurements, unless otherwise stated.

Results

In Figs. 1(a) and (b), the CD spectra of poly[Cys(CH_2COOH)] (a) and poly[Cys(C_2H_4COOH)] (b) are shown when various amounts of $CrCl_3$ are added. When $CrCl_3$ is not added, the CD spectra indicate the random coil state of respective polypeptides.3) The CD spectra change with the addition of $CrCl_3$; the residue ellipticity around 200 nm, $[\theta]_{200}$, changes from negative to positive and reaches as large as about 4×10^4 and 2×10^4 in the case of poly[Cys(CH_2COOH)] and poly[Cys(C_2H_4COOH)], respectively. The CD spectra at large values of C_M/C_p resemble those of the β-form in the case of poly[Cys(C_2H_4COOH)].3) In the case of poly[Cys(CH_2COOH)], however, the negative band around 225 nm shifts to 230 nm as C_M/C_p increases from 0.202 to 0.253. This shift of band position as well as a large residue ellipticity at 200 nm suggest a possible contribution from some electronic transition involving Cr^{3+} ions.

A charge transfer from a sulfur to Cr atom has been reported to exist around 240—260 nm.4—6) Peerce *et al.* reported an absorption band around 195 nm for a complex of Cr(III) with a ligand containing sulfur and nitrogen atoms.6) When a sulfur atom was substituted by a methylene group, this absorption was not found any more.6) It is likely that an electronic transition involving a thioether and Cr is present in this wavelength region. All these results suggest that the CD spectra of both polypeptides should be carefully interpreted when Cr^{3+} ions are bound to them.

Contrary to the case of $CrCl_3$, normal CD pattern is obtained for the β-forms induced by $FeCl_3$ or $LaCl_3$. The CD spectra in the presence of $FeCl_3$ are shown in Figs. 2(a) and (b).

In Figs. 3 and 4, the residue ellipticities at 200 nm are plotted against the mixing ratio for poly[Cys(CH_2COOH)] and poly[Cys(C_2H_4COOH)], respectively. As a comparison, the results obtained on Cu^{2+} ions in the case of poly[Cys(CH_2COOH)] and Cd^{2+} ions in the case of poly[Cys(C_2H_4COOH)] are also shown in these figures. These ions are the most effective species among divalent metal cations in the induction of the β-form of each polypeptide. To facilitate the comparison, the abscissa is expressed as C_s/C_p instead of C_M/C_p, where C_s represents the normality of various metal ions. The equivalent point is thus located at C_s/C_p=1, irrespective of the valences of ions. In the case of poly[Cys(CH_2COOH)], both Fe^{3+} and La^{3+} ions are less effective to induce the β-form than Cu^{2+}

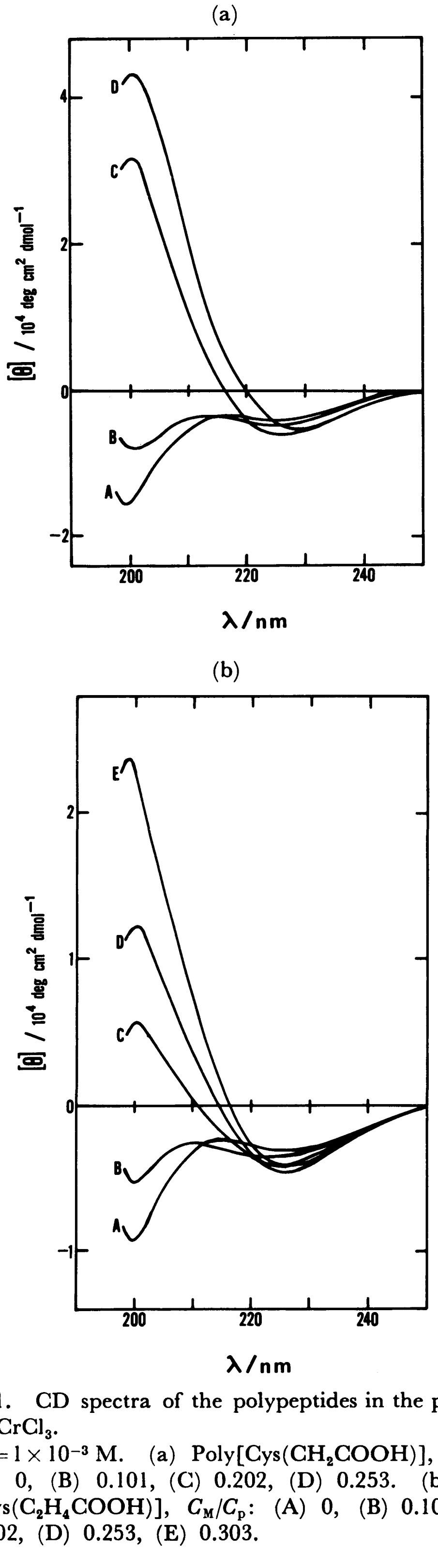

Fig. 1. CD spectra of the polypeptides in the presence of $CrCl_3$.
$C_p = 1 \times 10^{-3}$ M. (a) Poly[Cys(CH_2COOH)], C_M/C_p: (A) 0, (B) 0.101, (C) 0.202, (D) 0.253. (b) Poly-[Cys(C_2H_4COOH)], C_M/C_p: (A) 0, (B) 0.101, (C) 0.202, (D) 0.253, (E) 0.303.

ions. In the case of poly[Cys(C_2H_4COOH)] both Fe^{3+} and La^{3+} ions have a comparable power to or even stronger power than Cd^{2+} ions.

In Figs. 5(a) and (b), the state of poly[Cys(CH_2COOH)] solutions at various values of C_M/C_p is diagramatically shown, at 1 h (a) and 48 h (b) after the

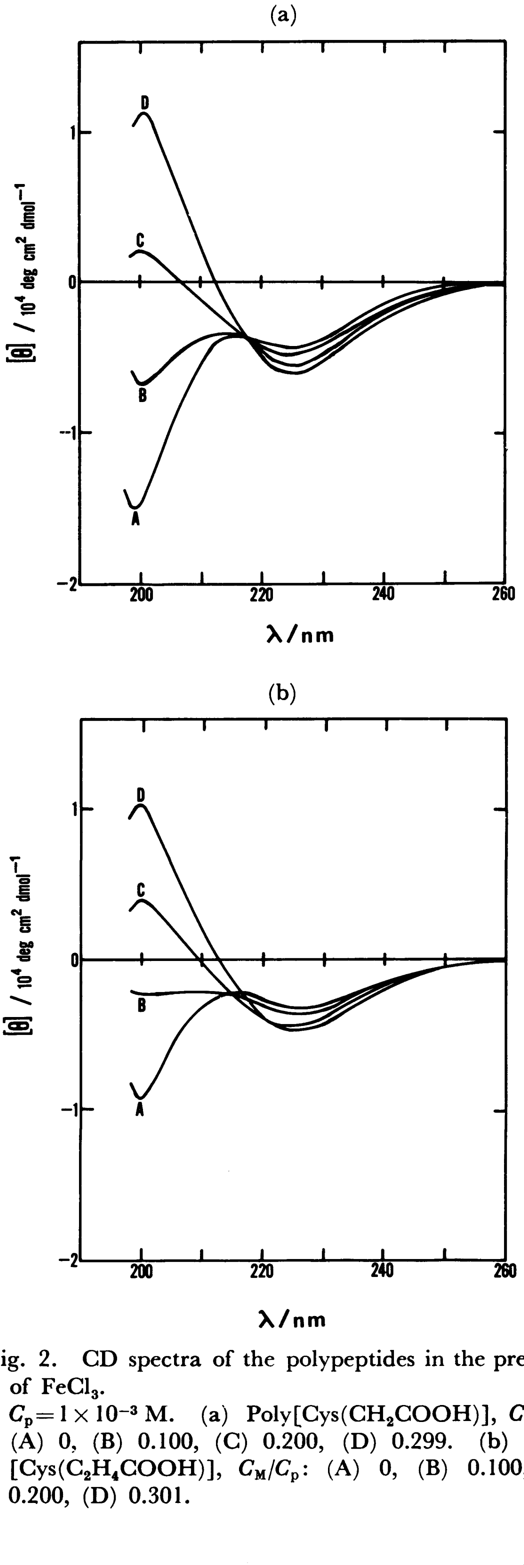

Fig. 2. CD spectra of the polypeptides in the presence of $FeCl_3$.
$C_p = 1 \times 10^{-3}$ M. (a) Poly[Cys(CH_2COOH)], C_M/C_p: (A) 0, (B) 0.100, (C) 0.200, (D) 0.299. (b) Poly-[Cys(C_2H_4COOH)], C_M/C_p: (A) 0, (B) 0.100, (C) 0.200, (D) 0.301.

addition of metal chlorides. In Figs. 6(a) and (b), corresponding results on poly[Cys(C_2H_4COOH)] are given. Slow precipitation occurs for $CrCl_3$ and $LaCl_3$ in the case of poly[Cys(CH_2COOH)], while it occurs only for $CrCl_3$ in the case of poly[Cys(C_2H_4COOH)]. Among three metal chlorides, the least solubility is found in $LaCl_3$ solutions for both polypeptides. In $CrCl_3$ and $LaCl_3$ solutions, poly[Cys(C_2H_4COOH)] is more soluble than poly[Cys(CH_2COOH)]. As discussed previ-

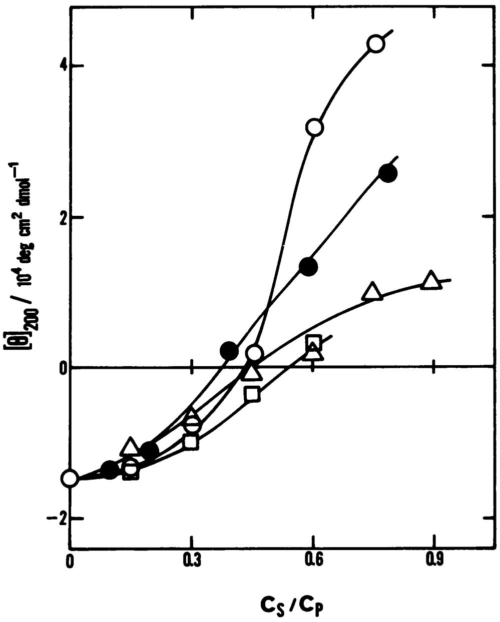

Fig. 3. Residue elliptidity of poly[Cys(CH_2COOH)] at 200 nm, $[\theta]_{200}$, as functions of metal chloride concentrations C_s.
$C_p = 1 \times 10^{-3}$ M. C_s is expressed in equiv dm^{-3}. (○) $CrCl_3$, (△) $FeCl_3$, and (□) $LaCl_3$. Previous results on $CuCl_2$ are shown by filled circles (●).

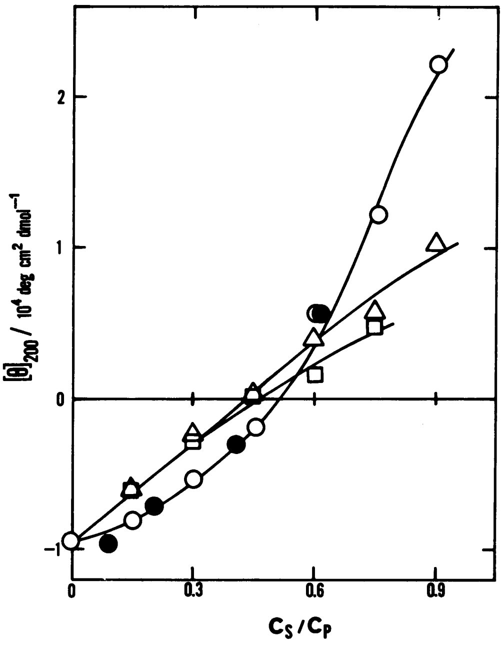

Fig. 4. Residue ellipticity of poly[C_2H_4COOH)] at 200 nm, $[\theta]_{200}$, as functions of metal chloride concentrations C_s.
$C_p = 1 \times 10^{-3}$ M. C_s is expressed in equiv dm^{-3}. (○) $CrCl_3$, (△) $FeCl_3$, and (□) $LaCl_3$. Previous results on $CdCl_2$ are shown by filled circles (●).

ously,2) the β-form of poly[Cys(C_2H_4COOH)] is always less soluble than that of poly[Cys(CH_2COOH)] when induced by the binding of proton or divalent cations. In this context, the reverse situation found in the present study on $CrCl_3$ and $LaCl_3$ is interesting but puzzling.

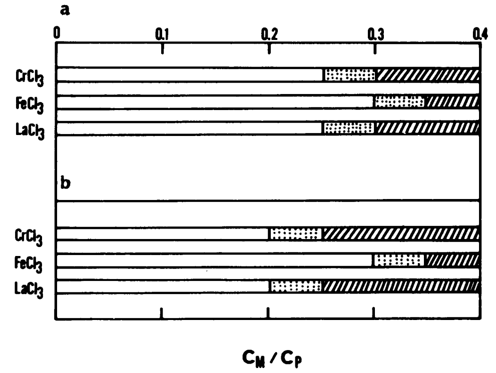

Fig. 5. Precipitation diagrams for aqueous solutions of poly[Cys(CH_2COOH)] in the presence of different metal chlorides.
$C_p = 1 \times 10^{-3}$ M. Blank region: clear solution. Dotted region: unspecified region. Shaded region: precipitation. (a) 1 h after preparation, and (b) 48 after preparation.

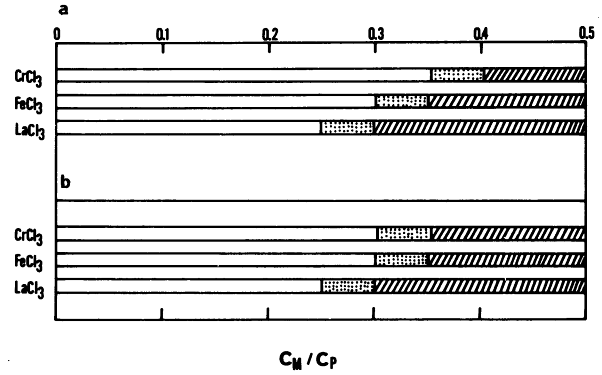

Fig. 6. Precipitation diagrams for aqueous solutions of poly[Cys(C_2H_4COOH)] in the presence of different metal chlorides.
$C_p = 1 \times 10^{-3}$ M. Blank region: clear solution. Dotted region: unspecified region. Shaded region: precipitation. (a) 1 h after preparation, and (b) 48 h after preparation.

Values of pH of the solutions of both polypeptides in the presence of the metal chlorides are shown in Fig. 7. The changes of pH are rather similar among three metal chlorides. This is in contrast to the result shown in Figs. 3 and 4, where a marked increase of $[\theta]_{200}$ is observed only for $CrCl_3$. The observed pH change supports the suggested interpretation that some contribution other than the conformational change is involved in the CD spectra of the solutions containing $CrCl_3$.

In Fig. 7, changes of pH take place at higher metal concentrations C_M in the case of poly[Cys(C_2H_4COOH)] as compared with the case of poly[Cys(CH_2COOH)]. This result implies that the binding of cations is stronger in poly[Cys(CH_2COOH)] than in poly[Cys(C_2H_4COOH)].

In Fig. 7, values of pH of the solutions of poly[Cys(C_2H_4COOH)] are higher by approximately one

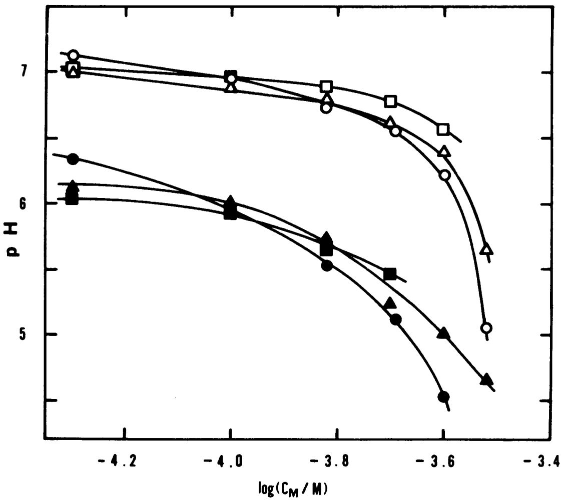

Fig. 7. Variation of pH with the logarithm of metal concentration C_M.
$C_p = 1 \times 10^{-3}$ M. Filled symbols refer to poly[Cys-(CH_2COOH)] and open symbols refer to poly[Cys-(C_2H_4COOH)]. $CrCl_3$ (●, ○), $FeCl_3$ (▲, △), and $LaCl_3$ (■, □).

pH unit than those of poly[Cys(CH_2COOH)]. This simply stems from different intrinsic dissociation constants K_0 of the carboxyl groups of the two polypeptides; pK_0=3.25 for poly[Cys(CH_2COOH)][7] and pK_0=4.00 for poly[Cys(C_2H_4COOH)].[8]

Time courses of $[\theta]_{200}$ after the addition of metal chlorides are shown in Figs. 8(a) and (d) for poly-[Cys(CH_2COOH)] and poly[Cys(C_2H_4COOH)], respectively. As shown in these figures, stationary values are reached within 5—6 h. Based on these results, all the data presented in the preceding sections were taken at 24 h (or longer) after the addition of metal chlorides, to confirm the equilibrium values.

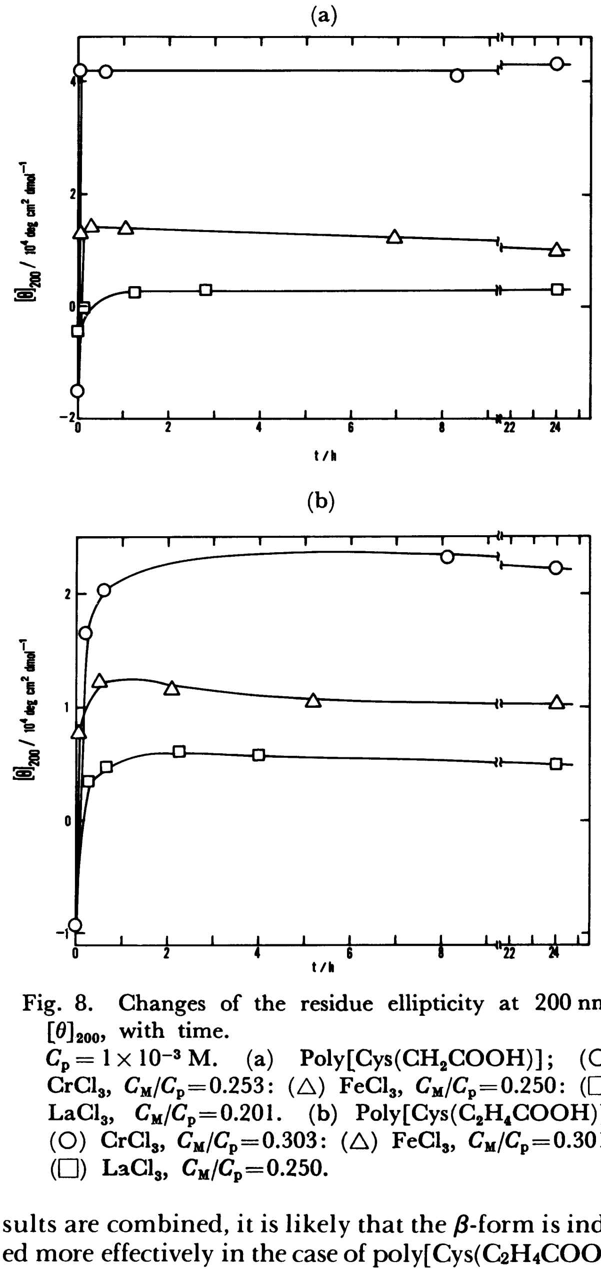

Fig. 8. Changes of the residue ellipticity at 200 nm, $[\theta]_{200}$, with time.
$C_p = 1 \times 10^{-3}$ M. (a) Poly[Cys(CH_2COOH)]; (○) $CrCl_3$, C_M/C_p=0.253: (△) $FeCl_3$, C_M/C_p=0.250: (□) $LaCl_3$, C_M/C_p=0.201. (b) Poly[Cys(C_2H_4COOH)]; (○) $CrCl_3$, C_M/C_p=0.303: (△) $FeCl_3$, C_M/C_p=0.301: (□) $LaCl_3$, C_M/C_p=0.250.

Discussion

Comparison of the Extents of the β-Form Induction between the Two Polypeptides. The total change of $[\theta]_{200}$ associated with the complete conversion is undoubtedly larger for poly[Cys(CH_2COOH)] than for poly[Cys(C_2H_4COOH)].[3,9] However, the magnitude of the total change has been definitely evaluated for none of these two polypeptides. This is one of the problems of comparing the extent of the β-form induction between these two polypeptides, which was discussed previously.[2] In the present study, however some conclusion can be derived on the effect of trivalent cations. Comparison of Fig. 3 with Fig. 4 shows that the values of $[\theta]_{200}$ of the two polypeptides are very similar at the same mixing ratio C_s/C_p for $FeCl_3$ and $LaCl_3$. The result suggests that the induction of the β-form occurs more extensively for poly[Cys(C_2H_4-$COOH$)] than for poly[Cys(CH_2COOH)] at the same value of C_s/C_p. On the other hand, the binding of counterions is suggested to occur more strongly for poly[Cys(CH_2COOH)] than for poly[(Cys(C_2H_4-$COOH$)] from the pH changes. When these two results are combined, it is likely that the β-form is induced more effectively in the case of poly[Cys(C_2H_4COOH)] than poly[Cys(CH_2COOH)], if compared at the same bound fraction of counterions. This situation is the same as that found for proton binding.[7]

Comparison of the Effect of the Trivalent Ions with That of the Divalent Ions. In the present study, all three kinds of trivalent ions induce the β-form of poly-[Cys(CH_2COOH)] and poly[Cys(C_2H_4COOH)] to approximately comparable extents with those found in the case of effective divalent cations. Counterion condensation of trivalent ions occurs more predominantly over divalent ions.[10] However, this cannot explain the effectiveness of the trivalent ions in the induction of the β-form. As described in the introduction part, the induction of the β-form occurs as a result of the annihilation of negative charges on the polypeptides by bound cations. However, the situation at the equivalent point is not uniquely determined but depends on the valency of bound counterions. A complete cancellation of charges takes place in the case of monovalent and divalent cations such as protons or Cu^{2+} ions. In contrast to these cases, complete cancellation between opposite charges cannot be expected in the case of trivalent ions, since a bound trivalent ion interacts most likely with two (or perhaps four) side chains, leaving a positive (or negative) charge. Under this situation, there would be a distribution of positive and negative charges on the pleated sheets, their sum being zero at the equivalent point. This supposed unique situation of trivalent ions would provide additional stability which is lacking in the case of monovalent and divalent counterions. Effective but rather nonspecific induction of the β-form found in the present study may be ascribed to this unique stabilization factor of trivalent ions.

The specificity of counterion species is expected to originate from the specificity involved in the interaction of carboxyl group with bound counterions. The precipitation of poly(acrylic acid) by various metal cations provides an interesting example. The effect of $LaCl_3$ on the precipitation of poly(acrylic acid) was similar to that of $MgCl_2$, $CaCl_2$, and $BaCl_2$ (designated as type L precipitation).[11] This behavior of $LaCl_3$ is in marked contrast with the present result. In the present study, $LaCl_3$ behaves like $CrCl_3$ and $FeCl_3$, quite differently from alkaline earth divalent cations, in the induction of the β-form of the polypeptides carrying carboxyl groups.

References

1) H. Maeda, J. Nakajima, K. Oka, K. Ooi, and S. Ikeda, *Int. J. Biol. Macromol.*, **4**, 352 (1982).

2) K. Oka, H. Maeda, and S. Ikeda, *Int. J. Biol. Macromol.*, **5**, 342 (1983).

3) S. Ikeda, A. Fukutome, T. Imae, and T. Yoshida, *Biopolymers*, **18**, 335 (1979).

4) C. J. Weschler and E. Deutsch, *Inorg. Chem.*, **12**, 2682 (1973).

5) R. H. Lane, F. A. Sedor, M. J. Gilroy, and L. E. Bennett, *Inorg. Chem.*, **16**, 102 (1977).

6) P. J. Peerce, H. B. Gray, and F. C. Anson, *Inorg. Chem.*, **18**, 2593 (1979).

7) H. Maeda and S. Ikeda, *Biopolymers*, **14**, 1623 (1975).

8) H. Maeda and S. Ikeda, *Biopolymers*, **10**, 2525 (1971).

9) H. Maeda and K. Ooi, *Biopolymers*, **20**, 1549 (1981).

10) F. Oosawa, "Polyelectrolytes," Marcel Dekker, New York (1971), Chap. 4.

11) A. Ikegami and N. Imai, *J. Polym. Sci.*, **56**,133 (1962).