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Effects of $Hg(ClO_4)_2$, $HgCl_2$, and $AgClO_4$ on the Conformations of Poly(*S*-carboxymethyl-L-cysteine) and Poly[*S*-(2-carboxyethyl)-L-cysteine] in Solution

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The effects of $Hg(ClO_4)_2$, $HgCl_2$, and $AgClO_4$ on the conformations of fully neutralized poly(*S*-carboxymethyl-L-cysteine) and poly[*S*-(2-carboxyethyl)-L-cysteine] in the absence of supporting electrolytes were examined by circular dichroism (CD) spectra. The induction of the β-form was suggested by the addition of $Hg(ClO_4)_2$ and $AgClO_4$, while it could not be concluded in the case of $HgCl_2$. Spectroscopic evidence for the coordination of metals with the two polypeptides was provided in the case of $Hg(ClO_4)_2$ and $HgCl_2$ by the appearance of a new CD band around 250—260 nm as well as the distortion of the polymer CD spectra in the region of 200—230 nm, while it was not obtained in the case of $AgClO_4$. The decrease of pH observed on addition of the three metal compounds also suggested the conformational change. Extraordinary lowering of pH and enhanced solubility were observed on addition of $HgCl_2$.

Recently, the effects of various bivalent metal ions on the conformations of poly(*S*-carboxymethyl-L-cysteine) (poly[Cys(CH_2COOH)])[1] and poly[*S*-(2-carboxyethyl)-L-cysteine](poly[Cys(CH_2CH_2COOH)])[2] were studied. Transition metal ions, such as Cu^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+} ions, effectively induced the β-form of these two polypeptides, while alkaline earth ions such as Mg^{2+}, Ca^{2+}, and Ba^{2+} ions 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 a kind of complex formation with counterions. In other words, shielding of the charged sites on the polypeptides by condensed counterions is not effective at all, even in the case of bivalent counterions, in the induction of the β-form of these two polypeptides. The complex formation with counterions is shown by ultraviolet absorption spectra and circular dichroism (CD) in the interactions of Cu^{2+} ions with these two polypeptides.[3] These two polypeptides have carboxyl and thioether groups in the side chains, which readily react with mercury and silver. According to the suggested mechanism of the β-form induction, these two metal species are expected to induce the β-form of the two polypeptides in solution.

In the present study, the effects of $Hg(ClO_4)_2$, $HgCl_2$, and $AgClO_4$ on the conformations of the two polypeptides are examined in their fully neutralized states mainly by CD.

Experimental

Poly[Cys(CH_2COOH)] (Lot No. KO125W; M_w=12000, DP=75) and poly[Cys(CH_2CH_2COOH)] (Lot No. H920WR; M_w=16000, DP=92) are the same samples as used in the previous studies.[1-3] Here M_w and DP denote, respectively, the weight average molecular weight and degree of polymerization. Reagent grade $HgCl_2$ and $AgClO_4 \cdot H_2O$ and $Hg(ClO_4)_2$ (99%) were purchased from Nakarai Chemicals Ltd. and were 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 cells of 1 and 5 mm light paths. 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 residue molarity, mol dm^{-3} (M) and is kept constant at 1×10^{-3} M in the present study. Metal concentrations C_M are expressed in molarity, mol dm^{-3} (M). The CD data are expressed in terms of the residue ellipticity [θ] based on the polymer concentration. To ascertain the time independent results, the solutions were kept at ambient temperature (25±2 °C) at least 20 h before the measurements were made.

In the present study, the two polymers were examined invariably in their fully neutralized states. Hence, they are abbreviated as poly[Cys(CH_2COONa)] and poly[Cys(CH_2CH_2-COONa)], except for the case that these abbreviations are confusing.

Results

I. $Hg(ClO_4)_2$ and $HgCl_2$. The CD spectra of poly[Cys(CH_2COONa)] in the presence of different amounts of $Hg(ClO_4)_2$ and $HgCl_2$ are shown in Figs. 1(a) and (b), respectively. In the absence of the mercury compounds, the CD spectrum of random coils at low ionic strengths is seen (curves A).[5] The CD spectra at large values of the mixing ratio C_M/C_p are different from those found on the mixtures of the β-form and random coils in the respect that a small positive CD band appears around 255 nm. This CD band is observed for both $Hg(ClO_4)_2$ and $HgCl_2$, and hence probably originates as a result of the coordination of Hg atoms to the side chains of the polypeptides. It is likely that the CD spectra in the wavelength region shorter than 230 nm are also modified. Nevertheless, the change of CD spectra by the addition of $Hg(ClO_4)_2$ can be ascribed mostly, if not all, to the induction of the β-form. The spectra of the β-forms of the two polypeptides are characterized by a negative band around 224 nm ($[\theta]\simeq-10^4$) and a positive band around 200 nm ($[\theta]\simeq3.5\times10^4$ for poly[Cys(CH_2COOH)][4] and $\simeq2\times10^4$ for poly[Cys(CH_2CH_2COOH)][5]). In the case of $HgCl_2$, definite conclusions cannot be obtained.

Residue ellipticities at 205 nm and 255 nm, $[\theta]_{205}$ and $[\theta]_{255}$, are plotted against the mixing ratio C_M/C_p in Fig. 2. The value of $[\theta]_{205}$ is tentatively chosen as a measure of the extent of the β-form induction, since the measurements at 200 nm was not always reliable due to the absorption of $HgCl_2$. The values of $[\theta]_{255}$ were mostly obtained using a cell of 5 mm light path. The

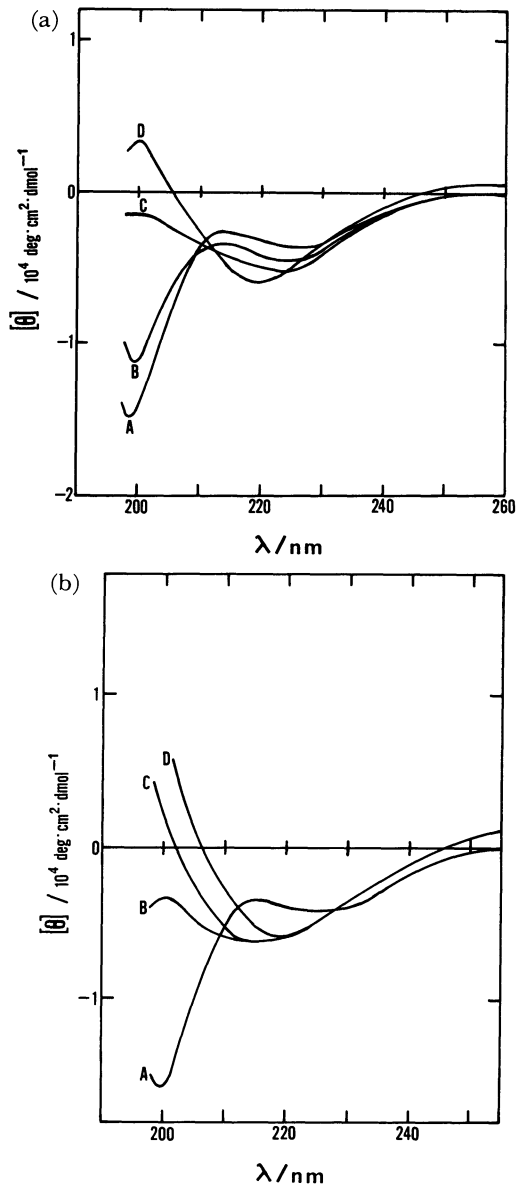

Fig. 1. CD spectra of poly[Cys(CH_2COONa)] in the presence of (a) $Hg(ClO_4)_2$ and (b) $HgCl_2$. Polymer concentration (C_p) is 1×10^{-3} M. Mixing ratios (C_M/C_p). (a); 0(A), 0.052(B), 0.156(C), and 0.259(D). (b); 0(A), 0.201(B), 0.402(C), and 1.005(D).

induction of the β-form is suggested for $Hg(ClO_4)_2$ also from the comparison with the previous results on $CuCl_2$,[1] which is given by a dashed curve in Fig. 2.

In Figs. 3(a) and (b), the CD spectra of poly[Cys-(CH_2CH_2COONa)] in the presence of different amounts of $Hg(ClO_4)_2$ and $HgCl_2$ are shown, respectively. In the case of $Hg(ClO_4)_2$, the spectrum at C_M/C_p= 0.415 resembles, in shape, that of the β-form except for the wavelength region larger than 240 nm. In the intermediate region of the mixing ratio, $0.15<C_M/C_p<0.26$, a characteristic CD pattern appears (curves C and D), which is different from the spectrum found on the mixtures of the β-form and random coils. The CD pattern found in the presence of $HgCl_2$ differs considerably from the spectrum characterizing the β-form of poly[Cys(CH_2CH_2COOH)] induced either by protonation or by the addition of bivalent metal

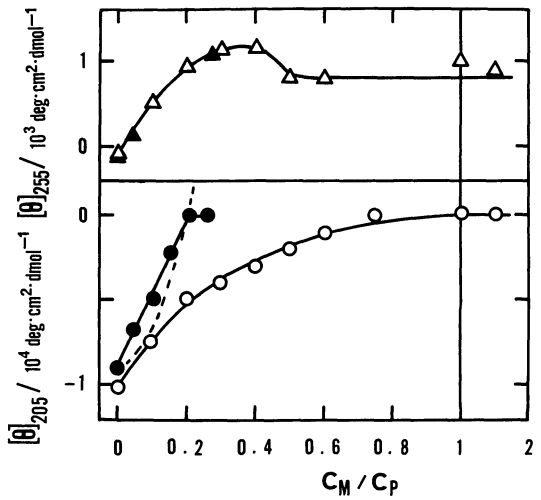

Fig. 2. Dependences of the residue ellipticities of poly[Cys(CH_2COONa)] at 205 nm ($[\theta]_{205}$) and 255 nm ($[\theta]_{255}$) on the mixing ratio (C_M/C_p). Open symbols (○, △) refer to $HgCl_2$ and filled symbols (●, ▲) refer to $Hg(ClO_4)_2$. A dashed curve represents the result on $CuCl_2$.

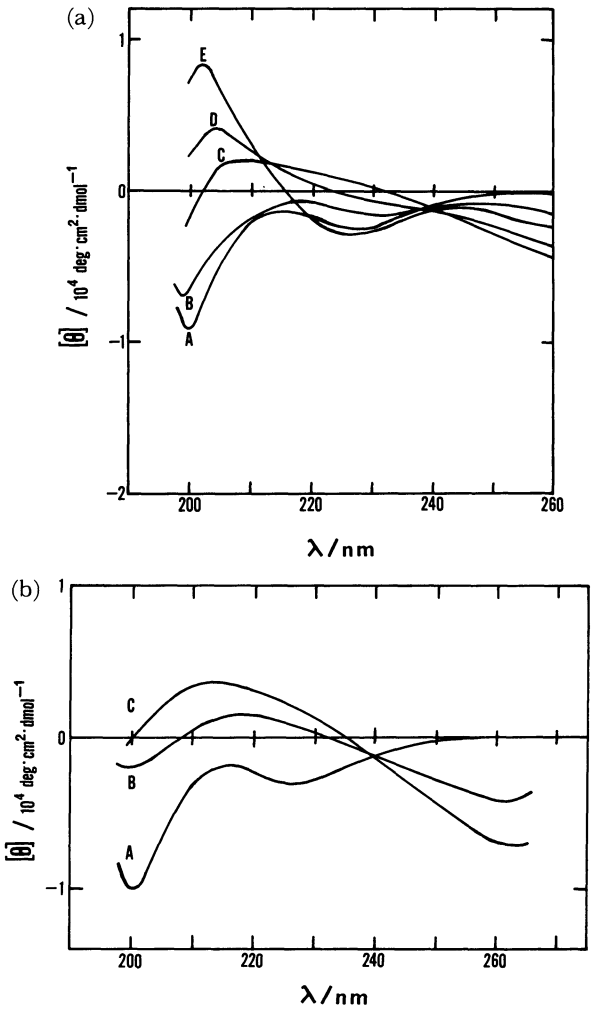

Fig. 3. CD spectra of poly[Cys(CH_2CH_2COONa)] in the presence of (a) $Hg(ClO_4)_2$ and (b) $HgCl_2$. Polymer concentration (C_p) is 1×10^{-3} M. Mixing ratios (C_M/C_p). (a); 0(A), 0.052(B), 0.156(C), 0.259(D), and 0.415(E). (b); 0(A), 0.100(B), and 0.200(C).

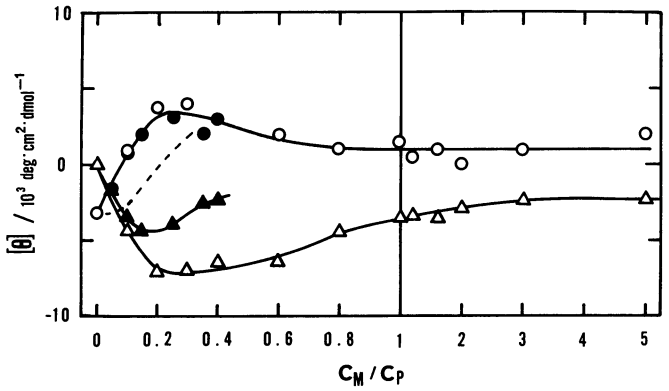

Fig. 4. Dependences of the residue ellipticities of poly[Cys(CH_2CH_2COONa)] on the mixing ratio (C_M/C_P).
210 nm (○, ●) and 260 nm (△, ▲). Open and filled symbols refer to $HgCl_2$ and $Hg(ClO_4)_2$, respectively. A dashed curve represents $[\theta]_{210}$ obtained on $CdCl_2$.

cations.[2] The CD spectra at the mixing ratios larger than about 0.2 fall between curves B and C. It is difficult to infer the induction of the β-form in the case of $HgCl_2$.

A large negative CD band appears around 260 nm for both $Hg(ClO_4)_2$ and $HgCl_2$, which becomes negligible at the wavelengths larger than 320nm. The residue ellipticities at 210 nm and 260 nm, $[\theta]_{210}$ and $[\theta]_{260}$, obtained at different mixing ratios are shown in Fig. 4 together with the previous results on $CdCl_2$[2] for 210 nm. The wavelength 210 nm is chosen partly because the characteristic CD pattern in the case of $HgCl_2$ shows the maximum around 210 nm and partly because reliable measurements of CD were hampered at 205 nm by strong absorption of $HgCl_2$. At 205 nm, CD spectra were obtained up to C_M/C_P=0.2 in the case of $HgCl_2$. The values of $[\theta]_{205}$ were very similar for both $Hg(ClO_4)_2$ and $HgCl_2$. Comparison of the present results with the results on $CdCl_2$ suggests the distortion of the polymer CD spectra as well as the induction of the β-form. It is likely that the β-form of poly[Cys(CH_2CH_2COOH)] is induced by the coordination of Hg atoms, at least, in the case of $Hg(ClO_4)_2$. The dependences of $[\theta]_{210}$ and $[\theta]_{260}$ on C_M/C_P are well correlated to each other, especially in the case of $HgCl_2$. This correlation also suggests that the induction of the β-form parallels the coordination of Hg atoms.

When the effects of $Hg(ClO_4)_2$ and $HgCl_2$ are compared between the two polypeptides, the characteristic CD patterns inherent to the coordination of Hg atoms are more evident in poly[Cys(CH_2CH_2COONa)] than in poly[Cys(CH_2COONa)]; a new CD band around 260 nm is more intense and the distortion of the CD spectra in the range 200—230 nm is more significant in poly[Cys(CH_2CH_2COONa)].

In Fig. 5, the dependences of pH on $Hg(ClO_4)_2$ and $HgCl_2$ concentrations (C_M) are shown in logarithmic scale for the two polypeptides. There is no appreciable difference between the effects of $Hg(ClO_4)_2$ (filled circles) and $HgCl_2$ (open circles). The profiles of pH change are very similar for the two polypeptides. The change of pH on log C_M is almost identical between the two polypeptides, when the pH values are corrected

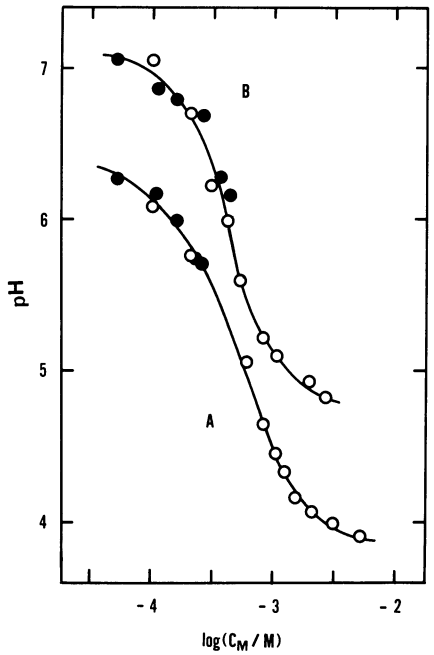

Fig. 5. Dependence of pH on the logarithm of the salt concentration (C_M).
Polymer concentration (C_P) is 1×10^{-3} M. (A) poly[Cys(CH_2COONa)] and (B) poly[Cys(CH_2CH_2COONa)]. Open and filled circles refer to $HgCl_2$ and $Hg(ClO_4)_2$, respectively.

for the difference in the intrinsic dissociation constants K_0; pK_0=3.2 for poly[Cys(CH_2COOH)][6] and 4.0 for poly[Cys(CH_2CH_2COOH)].[7] The correction is made in such a way that one of the two curves, either A or B, is shifted vertically by about 0.8 pH unit, which corresponds to the difference between the two values of pK_0. After the correction, these two curves are nearly identical to each other in the whole range of C_M examined. This profile of the pH change is , however, different from that expected for the effect of added salt on random coil polyelectrolytes.[8] The pH change thus suggests the conformational change. In the previous studies, pH decreased to about 4.5 on addition of the most effective species (Cu^{2+} ions) to poly[Cys(CH_2COONa)][1] while pH decreased to about 6.0 in the case of poly[Cys(CH_2CH_2COONa)][2]. In the present study, the extents of pH decrease by the addition of $HgCl_2$ are much larger than those found in previous studies, while the effects of $Hg(ClO_4)_2$ are consistent with previous data. The extraordinary lowering of pH on addition of $HgCl_2$ may suggest the coordination of $HgCl_2$ to peptide nitrogens, which releases hydrogen ions and converts the partially formed β-form to random coils.

A marked difference between $Hg(ClO_4)_2$ and $HgCl_2$ is found in Figs. 2, 4, and 5 concerning the solubilities of the two polypeptides. Precipitation of the two polypeptides did not occur at the mixing ratio of 5 in the case of $HgCl_2$. Precipitation occurred at the mixing ratios of about 0.3 for poly[Cys(CH_2COONa)] and about 0.4 for poly[Cys(CH_2CH_2COONa)] in the case of $Hg(ClO_4)_2$. The solubilities found in the case of $Hg(ClO_4)_2$ are consistent with the previous results on other bivalent metal cations, although they are the least or nearly the least among the solubilities in the

presence of bivalent cations.[1,2] It is interesting to note that the solubility in the presence of $Hg(ClO_4)_2$ is larger for poly[Cys(CH_2CH_2COONa)] than for poly[Cys(CH_2COONa)].

In conclusion, the induction of the β-form occurs on addition of $Hg(ClO_4)_2$ to the solutions of poly[Cys(CH_2COONa)] or poly[Cys(CH_2CH_2COONa)] due to the formation of a complex between them, as suggested by the appearance of a new CD band around 250—260 nm. In the case of $HgCl_2$, on the other hand, the induction of the β-form cannot be concluded, although evidence for the complex formation is obtained.

II. $AgClO_4$. In Figs. 6(a) and (b), the CD spectra of poly[Cys(CH_2COONa)] and poly[Cys(CH_2CH_2COONa)] are shown respectively in the presence of different amounts of $AgClO_4$. The change of CD spectra indicates the formation of the β-form by the addition of $AgClO_4$. This result is the first example of the β-form of these two polypeptides induced *in solution* by a univalent metal cation. Based on the mechanism suggested for the induction of the β-form of these two polypeptides deduced from previous studies, silver ions are supposed to be covalently bound to the side chains of the polypeptides. However, there is no new CD band in the whole wavelength region examined nor anomalous pattern around 200—230 nm, contrary to the case found on the addition of the mercury compounds.

In Fig. 7, the residue ellipticities at 200 nm, $[\theta]_{200}$, are shown as functions of the mixing ratio. The results on

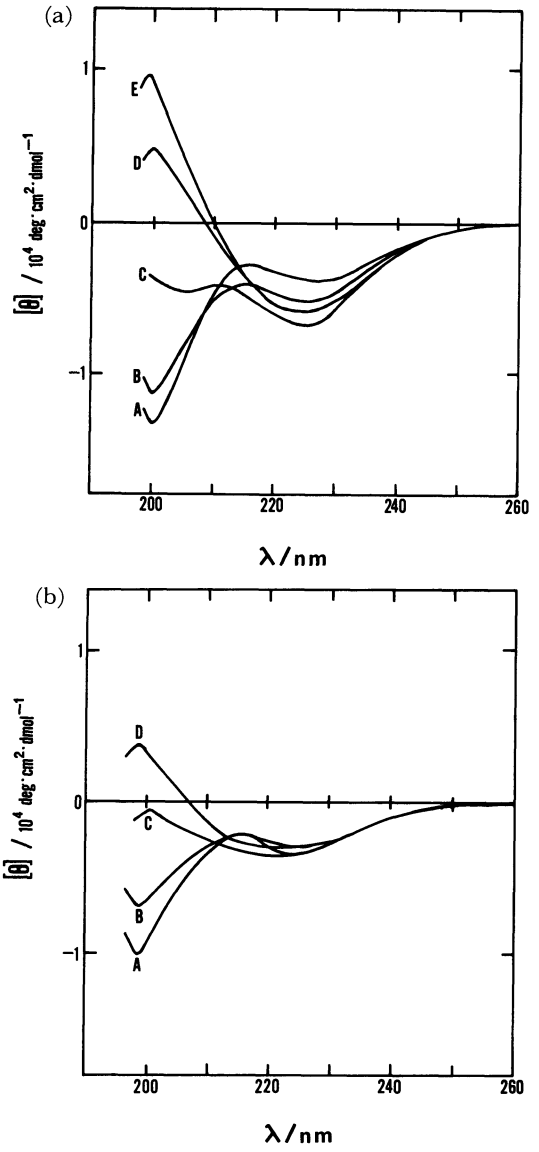

Fig. 6. CD spectra of (a) poly[Cys(CH_2COONa)] and (b) poly[Cys(CH_2CH_2COONa)] in the presence of $AgClO_4$.
Mixing ratios (C_M/C_P). (a); 0(A), 0.101(B), 0.402(C), 0.503(D), and 0.754(E). (b); 0(A), 0.102(B), 0.409(C), and 0.512(D).

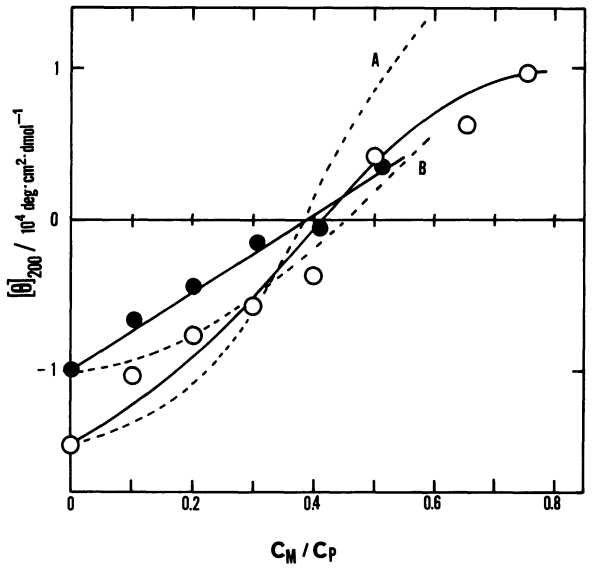

Fig. 7. Dependence of the residue ellipticity at 200 nm, $[\theta]_{200}$, on the mixing ratio (C_M/C_P).
(○) Poly[Cys(CH_2COONa)] and (●) Poly[Cys(CH_2CH_2COONa)].
Polymer concentration (C_P) is 1×10^{-3} M. Dashed curves represent the results on (A) $CuCl_2$–poly[Cys(CH_2COONa)] and (B) $CdCl_2$–poly[Cys(CH_2CH_2COONa)]. The scale of abscissa is doubly enlarged for these dashed curves.

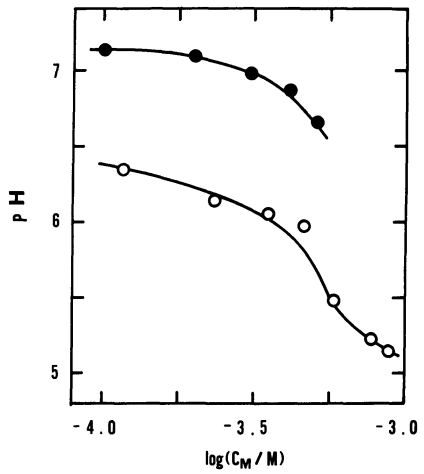

Fig. 8. Dependence of pH on the logarithm of $AgClO_4$ concentration (C_M).
(○) Poly[Cys(CH_2COONa)] and (●) poly[Cys(CH_2CH_2COONa)]. Polymer concentration (C_P) is 1×10^{-3} M.

$CuCl_2$ for poly[Cys(CH_2COONa)] (curve A)[1] and on $CdCl_2$ for poly[Cys(CH_2CH_2COONa)] (curve B)[2] are also shown. These are the most effective inducers of the β-forms of respective polypeptides in the previous studies. To facilitate the comparison, the scale of the abscissa for these bivalent cations is doubly enlarged. The induction of the β-form by $AgClO_4$ is comparable with $CdCl_2$ in the case of poly[Cys(CH_2CH_2COONa)], while it is less effective than $CuCl_2$ in the case of poly[Cys(CH_2COONa)].

In Fig. 8, values of pH of the solutions of the two polypeptides are given as functions of $AgClO_4$ concentration (C_M) in the logarithmic scale. As in the case of the mercury compounds shown in Fig. 5, the changes are nearly identical for the two polypeptides, when either of the two curves is shifted vertically by about 0.8 pH unit, the correction for the difference in the intrinsic dissociation constants. The decrease of pH is much larger than that expected for the effect of added salt on random coil polyions and hence confirms the coordination of Ag^+ ions, probably, to the side chains.

Precipitation occurred at the mixing ratios of about 0.8 for poly[Cys(CH_2COONa)] and 0.5 for poly[Cys(CH_2CH_2COONa)], which correspond to 0.4 and 0.25 in the case of bivalent ions, respectively. They are comparable with those found on $Hg(ClO_4)_2$, 0.3 and 0.4 given above. The solubility of poly[Cys(CH_2CH_2COONa)] in the presence of $AgClO_4$ is lower than that of poly[Cys(CH_2COONa)], which is consistent with the general trend found for the β-forms of these two polypeptides induced by bivalent metal cations and hydrogen ions.[1,2]

Discussion

In the present study, new CD band appears in the region of 240—260 nm and distortion of the CD spectra occurs in the region of 200—220 nm on addition of $Hg(ClO_4)_2$ or $HgCl_2$ to the solutions of poly[Cys(CH_2COONa)] and poly[Cys(CH_2CH_2COONa)]. These changes can be ascribed to the electronic transition involving Hg atoms. The absorption of $HgCl_2$ is more intense than that of $Hg(ClO_4)_2$ in the region of 200—260 nm; the molar absorption coefficients differ by about one order around 230 nm.[9] The absorption maxima are about 205 nm for $HgCl_2$ and below 190 nm for $Hg(ClO_4)_2$.[9] This can explain why the anomaly was observed more clearly for $HgCl_2$ than for $Hg(ClO_4)_2$. In the case of $AgClO_4$, which has absorption bands around 225, 210, and 194 nm,[10] neither distortion of the CD spectra occurs nor new CD band appears appreciably, although the β-form is induced.

The different solubilities of the two polypeptides are seen from Figs. 2, 4, and 5 between the solutions containing $Hg(ClO_4)_2$ or $HgCl_2$. The difference may be related to the different extents of the covalent nature of these two compounds. While $Hg(ClO_4)_2$ readily dissociates, $HgCl_2$ remains mostly undissociated in aqueous solutions. The induction of the β-form, the low solubility, and the characteristic decrease of pH are reasonably understood in the case of $Hg(ClO_4)_2$ in terms of the interactions of ionic species Hg^{2+} with the polypeptides. Contrary to the expected behavior for neutral species, the complex formation is indicated from the CD spectra in the case of $HgCl_2$. [Figs. 1(b) and 3(b)]. It is difficult, however, to infer the induction of the β-form from the CD spectra.

All the obtained data (CD spectra, pH change, and solubility) in the case of $HgCl_2$ cannot be consistently explained by assuming for the bound species either neutral species or bivalent ionic species. Explanations in terms of various possible bound species could be developed. However, it is not pertinent to present highly speculative discussions here.

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