Circular Dichroism and the pH-Induced β-Coil Transition of Poly(S-Carboxymethyl-L-Cysteine) and Its Side-Chain Homolog

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Synopsis

The CD of aqueous solutions of poly(S-carboxymethyl-L-cysteine) and poly(S-carboxyethyl-L-cysteine) has been measured at different pH, and the pH-induced β-coil transition is observed by changes in residue ellipticity of dichroic bands around 200 and 225 nm. The residue ellipticity at 200 nm of the former polypeptide is twice as large as that of the latter, when the β-conformation is formed in solution. However, the β-conformation of the latter polypeptide is more stable against electrostatic repulsion than that of the former. The transition curve of poly(S-carboxymethyl-L-cysteine) has also been determined for different molecular weights. The curves were found to be completely coincident with one another if the degree of polymerization were higher than about 100. Such a transition curve is generally divided into three steps: initiation, cooperative formation, and rearrangement of hydrogen bonds. The cooperative step is very sharp, occurring at a constant pH. These steps become agglomerated into two or one when the polypeptide concentration or added salt concentration is increased.

INTRODUCTION

Although the CD of α-helical polypeptides is well characterized by a strong positive band at 191 nm and two negative bands at 209 and 222 nm,[1–6] it is known that the CD of β-form polypeptides is different from that of α-helical polypeptides and has a positive band around 200 nm and a weaker negative band around 217 or 225 nm. Fasman and coworkers[7,8] classified the β-conformation of polypeptides into two groups, I-β and II-β forms, on the basis of the location of the trough of optical rotatory dispersion or of the CD band at the longest wavelength transition, i.e., at the n-π^* transition.

The I-β form shows CD having a positive band around 196–200 nm and a negative band around 216–220 nm. The β-form poly(L-lysine) formed at alkaline pH by heat treatment belongs to this type.[9–11] Some of the higher oligo(L-alanine)s[12] and oligo(L-isoleucine)s[13] were found to be in the I-β form in trifluoroethanol, and decaalanine polyoxyethylene ester[14] can also assume the same form in both trifluoroethanol and water. On the other hand, poly(S-carboxymethyl-L-cysteine) (poly[Cys(CH_2COOH)]) and poly(S-carboxyethyl-L-cysteine) (poly[Cys($(CH_2)_2COOH$)]) have been shown to belong to the II-β form,[8,15,16] which gives CD having a negative

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band around 225 nm. Water-soluble poly(L-serine)[17,18] and low-molecular-weight poly(γ-benzyl-L-glutamate)[19] can also form the II-β form in aqueous alcoholic mixture and organic solvents, respectively. Above a certain critical concentration, heat treatment of poly(L-glutamic acid) at acid pH also produces conformations called β_1 and β_2 forms, which are more similar to the II-β form than to the I-β form.[20]

Poly(L-tyrosine) has been shown to undergo a pH-induced β-coil transition in water,[21,22] and its conformation has been investigated by means of various methods.[23-25] Owing to phenol chromophores on its side chain, the CD of peptide groups is obscured in the region of the peptide transition.

In the present work, we will investigate the CD of poly[Cys(CH_2COOH)] and poly[Cys((CH_2)$_2$COOH)] in water down to 186 nm and observe the pH-induced β-coil transition as revealed by CD. Specifically, the differences in CD between the two polypeptides and in the transition behavior between them are noted in detail. Furthermore, several samples of poly-[Cys(CH_2COOH)] of different molecular weights are prepared, and we will examine the effects of molecular weight on the CD and β-coil transition and show the presence of a critical molecular weight for the occurrence of definite transition behavior.

EXPERIMENTAL

Materials

Poly[Cys(CH_2COOH)] was prepared by the method given previously.[26] Debenzylation of poly(S-carbobenzoxymethyl-L-cysteine) was mostly performed by the addition of acetic acid solution saturated with HBr to the polymerization mixture or to the solid polymer. Poly[Cys((CH_2)$_2$-COOH)] was prepared by the previous method.[16] The intrinsic viscosity of the aqueous solution in 0.2*M* NaCl at pH 7.0 was measured by an Ubbelohde viscometer at 25°C, and the molecular weight of the samples was tentatively estimated from this value, assuming the intrinsic viscosity–molecular weight relation for poly(L-glutamic acid) in the same solvent.[27] The values of intrinsic viscosity, molecular weight as the acid form, and degree of polymerization (*DP*) of the polypeptides are given in Table I.

Measurements

To make up a stock solution of known concentration, a weighed amount of poly[Cys(CH_2COOH)] was dissolved in an alkaline solution, of which the NaOH concentration was less than 0.01*N*. For most experiments, the stock solution of about 0.1 g/dl was brought to a desired pH by adding 0.01*N* HCl or 0.01*N* NaOH, and diluted to about 0.02 g/dl or $10^{-3}N$ by the addition of distilled water or a solution of $NaClO_4$. Poly[Cys((CH_2)$_2$COOH)] was initially dissolved in water, if it was the sodium salt, and then the solution was brought to a desired pH and diluted in a similar way. The pH

TABLE I
Intrinsic Viscosity and Molecular Weight of Poly[Cys(CH_2COOH)] and Poly[Cys((CH_2)$_2$COOH)]

Polymer	Sample Code	$[\eta]$ (dl/g)[a]	$\overline{M}_w$[b]	*DP*	Debenzylation
Poly[Cys(CH_2COOH)]	F62-0-A	0.612	28,000	174	c
	K1201	0.497	22,600	140	d
	F62-0-B	0.443	20,000	124	c
	E602	0.230	10,100	63	d
	E527	0.089	3800	24	d
	F57-0-A	0.050	2100	13	e
	E515	0.041	1700	11	d
Poly[Cys((CH_2)$_2$COOH)]					
	K917	0.310	13,800	79	e
	L111	0.106	4550	26	e

[a] Intrinsic viscosity measured in 0.2*M* NaCl, pH 7, at 25°C.

[b] Molecular weight for the acid form calculated by the equation (Ref. 27) $[\eta] = 3.13 \times 10^{-5} \overline{M}_w^{0.965}$.

[c] Addition of acetic acid solution saturated with HBr to the polymerization mixture in CH_2Cl_2.

[d] Addition of acetic acid solution saturated with HBr to the solid benzyl ester.

[e] Passage of HBr gas through the polymerization mixture in CH_2Cl_2 after diluted with an equal volume of $CHCl_3$.

of the solutions was measured with a Hitachi-Horiba F5-X or $F7_{SS}$ pH meter, calibrated with standard buffers of pH 4.00 and 6.88.

Ultraviolet absorption spectra were measured on a Jasco J-5 (ORD/UV 5) spectropolarimeter having a 450-W xenon lamp as light source and a quartz cell of 1-mm pathlength. CD was measured on a Jasco J-20 circular dichrometer over the wavelength region from 300 to 186 nm at room temperature (25°C). The dichrometer was calibrated by means of D-10-camphor sulfonic acid, as previously described.[28] Quartz cells of 1- and 0.058-mm pathlengths were used.

Values of the residue extinction coefficient, ϵ, are given in the units of l. mol^{-1} cm^{-1}, and values of the residue ellipticity, $[\theta]$, are in deg cm^2 $dmol^{-1}$.

RESULTS

Differences between the Two Polypeptides

Both poly[Cys(CH_2COOH)] and poly[Cys((CH_2)$_2$COOH)] in water undergo pH-induced transitions from the random coil to the β-conformation. They are in the β-conformation at lower pH, while they are in the random coil at alkaline pH. It was shown from optical rotatory dispersion, viscosity, and ir spectra that the transition pH of poly[Cys(CH_2COOH)] in water was about 4.83 at a concentration of 0.6 g/dl.[25,26] Similarly, the transition pH of poly[Cys((CH_2)$_2$COOH)] was found to be around 5.8.[16] The difference in the transition pH of the two polypeptides has been at-

TABLE II
Residue Extinction Coefficient of Poly[Cys(CH_2COOH)] and Poly[Cys((CH_2)$_2$COOH)] in Water

Polymer	Random Coil			β-Conformation		
	pH	λ (nm)	ε	pH	λ (nm)	ε
Poly[Cys(CH_2COOH)] (K1201)	7.11	190	13,000	4.68	190	13,500
Poly[Cys((CH_2)$_2$COOH)] (L111)	6.52	190	8700	5.32	190	10,000

tributed to the different pK_{int} values of the side-chain carboxyl groups, 3.0 and 4.0.[29-31]

Figure 1 illustrates far-uv absorption spectra of the two polypeptides at acid pH, where they are in the β-conformation. The absorption band located at 190 nm can be assigned to the π-π^* transition of the peptide groups.[32] Although the location of the band remains unaltered by the change in pH or polypeptide conformation, its residue extinction coefficient shows a subtle change with pH, as given in Table II. The β-conformation is slightly hyperchromic as compared with the random coil, and this observation is similar to the case of poly(L-lysine).[33] Poly[Cys(CH_2COOH)] absorbs more strongly than poly[Cys((CH_2)$_2$COOH)].

Figures 2 and 3 show the typical CD spectra of the two polypeptides at different pH. At alkaline pH, where the polypeptide chains are randomly coiled, the spectra of both polypeptides have two negative bands at 198 and 225–228 nm. They are not significantly dependent on pH, when the pH is higher than about 6 for poly[Cys(CH_2COOH)] and about 7.4 for poly-[Cys((CH_2)$_2$COOH)]. At acid pH, where the polypeptides are mostly in the β-conformation, both polypeptides exhibit CD, having a positive band at 200 nm and a negative band at 225 nm, which is characteristic of the II-β form. The solution becomes turbid when its pH is lowered below 3.9 for

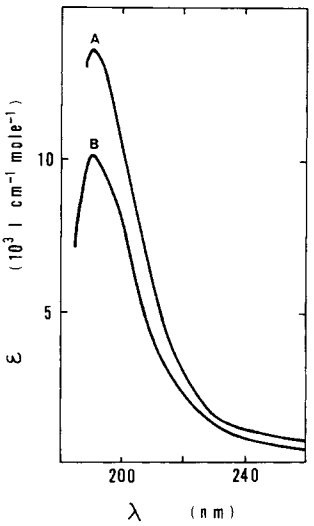

Fig. 1. Absorption spectra of poly[Cys(CH_2COOH)] (K1201), 0.0099 g/dl, at pH 4.68 (curve A); and poly[Cys((CH_2)$_2$COOH)] (L111), 0.0175 g/dl, at pH 5.32 (curve B).

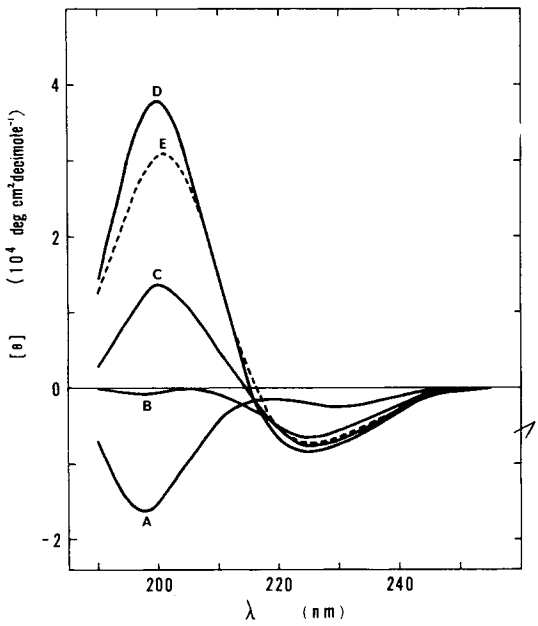

Fig. 2. CD of poly[Cys(CH_2COOH)] (K1201) in water, 0.0195 g/dl or $1.21 \times 10^{-3}N$, at different pH: A, 7.41; B, 5.56; C, 5.39; D, 3.94; E, 3.14. Broken curve is for a turbid solution.

poly[Cys(CH_2COOH)] and 5.9 for poly[Cys((CH_2)$_2$COOH)]. The largest residue ellipticity of the positive dichroic band is attained at the most acid pH where the solution is still clear, but its magnitude is about twice as large for poly[Cys(CH_2COOH)] as for poly[Cys((CH_2)$_2$COOH)].

We could not find any isodichroic point on the CD spectra effective over the whole region of β-coil transition, but it would be possible to assign isodichroic points at each step if the transition is divided into a few steps. In the pH-induced β-coil transition of poly(L-tyrosine) in water, isosbestic points on the absorption spectra in the near-uv region are manifest at each of two steps.[25] A clear isodichroic point exists at 204 nm for the CD of poly(L-glutamic acid) and poly(L-lysine) during the helix–coil transition.[4,34] However, the CD spectra of poly(L-glutamic acid) cease to pass the isodichroic point at low pH, and this was attributed to the aggregation of poly(L-glutamic acid) helices.[35]

The location and magnitude of the CD bands for the two polypeptides in the random coil and β-conformation are given in Table III. It is evident that the negative CD band around 225 nm is assignable to the n-π^* transition, and the band around 200 nm is associated with the π-π^* transition of the peptide groups.[36] Differences in residue ellipticity between the two polypeptides in the random coil would reflect different modes of the electronic coupling of peptide groups with side-chain chromophores, as are also revealed in the difference in residue extinction coefficient for the π-π^* transition. Table IV gives the values of the ratio of residue ellipticity to residue extinction coefficient, $[\theta]_{\sim 200}/\epsilon_{190}$. It is seen that the dissymmetry factors are almost equal to each other in the random-coil form.

TABLE III
Residue Ellipticity of Poly[Cys(CH_2COOH)] and Poly[Cys((CH_2)$_2$COOH)] in Water

Polymer	Random Coil			β-Conformation		
	pH	λ (nm)	[θ]	pH	λ (nm)	[θ]
Poly[Cys(CH_2COOH)]	7.25	228	−2500	3.94	225	−8500
(K1201)		198	−16,000		200	38,000
Poly[Cys((CH_2)$_2$COOH)]	7.91	225	−4100	5.94	225	−8200
(K917)		198	−12,000		200	18,400

When the polypeptides are in the β-conformation, the residue ellipticity for the π-π* transition of poly[Cys(CH_2COOH)] is twice as large as that of poly[Cys((CH_2)$_2$COOH)], and the corresponding dissymmetry factor is about 1.5 times as large. This suggests that the two polypeptides have different modes of main-chain folding or side-chain conformation in the β-conformation.

Figure 4 shows plots of the residue ellipticity of the band around 200 nm vs pH for the two polypeptides. The β-coil transition is sharp at pH 5.5 for poly[Cys(CH_2COOH)] and pH 7.3 for poly[Cys((CH_2)$_2$COOH)]. It may be observed that the transition pH differs by 1.8 pH units for the two side-chain homologs, whereas the difference in the pK_{int} values of the side-chain carboxyl groups has been known to be about 1 pH unit. During the charging of the random-coil conformation, the electrostatic interactions would not differ very much for the two polypeptides. Thus, we must

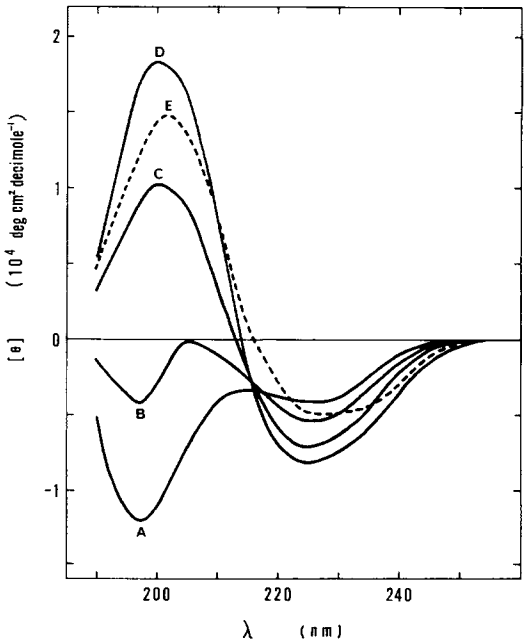

Fig. 3. CD of poly[Cys((CH_2)$_2$COOH)] (K917) in water, 0.0205 g/dl or 1.17 × $10^{-3}N$, at different pH: A, 7.91; B, 7.32; C, 6.94; D, 5.94; E, 4.50. Broken curve is for a turbid solution.

TABLE IV
Dissymmetry Factor and Rotatory Strength for the n-π^* and π-π^* Transitions

Polymer	Transition	Random Coil		β-Conformation	
		$[\theta]_{\sim 200}/\epsilon_{190}$	R (DBM)	$[\theta]_{\sim 200}/\epsilon_{190}$	R (DBM)
Poly[Cys(CH_2COOH)]	n-π^*		−0.022		−0.075
	π-π^*	−1.23	−0.107	2.82	0.253
Poly[Cys((CH_2)$_2$COOH)]	n-π^*		−0.031		−0.078
	π-π^*	−1.38	−0.081	1.84	0.122

conclude that the β-conformation of poly[Cys((CH_2)$_2$COOH)] has greater stability relative to the random coil when compared with that of poly-[Cys(CH_2COOH)]. Previously, the transition pH was thought to differ by about 1 pH unit, corresponding to the shift of pK_{int} value, for the two polypeptides. This conclusion probably resulted from observations with a lower molecular weight sample of poly[Cys((CH_2)$_2$COOH)] and at higher concentrations.

If the observed β-coil transition is regarded as a conformational change from the uncharged β-form to the charged random coil, the free energy of transition, $\beta^{\circ} \rightarrow c^- + H^+$, can be divided into the free energy change from the uncharged β-form to the random coil, $\beta^{\circ} \rightarrow c^{\circ}$, and the work for ionizing the random coil, $c^{\circ} \rightarrow c^- + H^+$. The latter work is composed of the work to dissociate a hydrogen ion from an isolated carboxyl group and the electrostatic free energy of the charged random coil. When the free energy of transition, $\Delta G_{\beta^{\circ} \rightarrow c^- + H^+}$, is measured by the transition pH, pH_t, its difference (per residue) between the two polypeptides is given by

$$\Delta G_{\beta^{\circ} \rightarrow c^- + H^+}(\mathrm{Et}) - \Delta G_{\beta^{\circ} \rightarrow c^- + H^+}(\mathrm{Me}) = 2.30\, RT[pH_t(\mathrm{Et}) - pH_t(\mathrm{Me})]$$
$$= 2450 \text{ cal/mol}$$

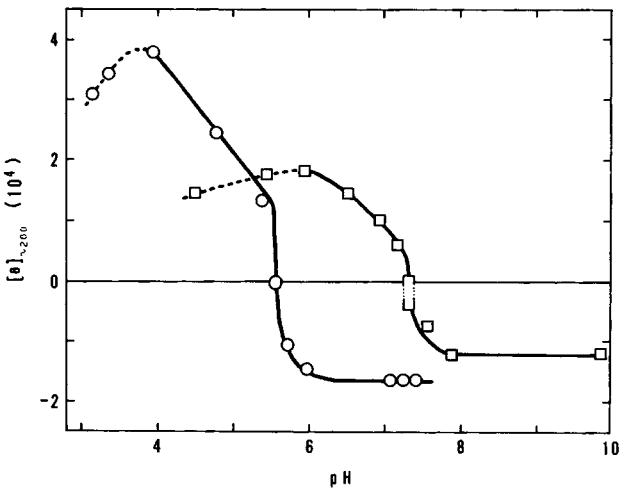

Fig. 4. The pH-induced transitions of poly[Cys(CH_2COOH)] (K1201) (○) and poly-[Cys((CH_2)$_2$COOH)] (K917) (□) in water. Dashed parts indicate that the solutions are turbid.

where (Me) and (Et) stand for poly[Cys(CH_2COOH)] and poly-[Cys((CH_2)$_2$COOH)], respectively. Since the negative intrinsic affinity of the side-chain carboxyl group with the hydrogen ion differs by

$$\Delta G^0_{int}(\text{Et}) - \Delta G^0_{int}(\text{Me}) = 2.30\,RT[\text{p}K_{int}(\text{Et}) - \text{p}K_{int}(\text{Me})] = 1360 \text{ cal/mol}$$

the intrinsic stability of the uncharged β-conformation relative to the uncharged random coil of poly[Cys((CH_2)$_2$COOH)] is higher than that of poly[Cys(CH_2COOH)] by

$$\Delta G_{\beta^\circ \rightarrow c^\circ}(\text{Et}) - \Delta G_{\beta^\circ \rightarrow c^\circ}(\text{Me}) = 1090 \text{ cal/mol}$$

provided that the electrostatic free energies of the charged random coils are equal to each other in the two polymers.

Stepwise β-Coil Transition

Although high-molecular-weight samples of poly[Cys(CH_2COOH)] can form the β-conformation, it has been shown that a low-molecular-weight sample (E515) cannot be transformed into the β-conformation by changing pH.[15,26,29] Even the two different samples of high molecular weight (E527 and E602) showed some small differences in optical rotatory dispersion and titration behavior.

Figure 5 shows the transition profiles of poly[Cys(CH_2COOH)] of different molecular weights, as revealed in the change in CD. It is seen that the transition behavior is dependent on molecular weight, when it is low. As the molecular weight increases, the β-conformation is more stable against electrostatic repulsion, and the transition becomes sharper.

When the *DP* is higher than about 50, the CD of the random-coil form is coincident for all the polypeptide samples, indicating that the random-coil conformation is free from the terminal or end effect. When the *DP* is higher than about 100, the transition behavior becomes independent of molecular weight of the samples. Then a very sharp transition step at a single pH, 5.5, and a less sharp step in a lower region are distinct. The β-conformation formed at the lowest pH, 3.9, in water would be the most perfect one stable in solution. The β-conformation is free from the periphery or edge effect when each constituent polypeptide chain consists of more than about 100 residues.

It can be seen that the pH-induced conformational change of poly[Cys-(CH_2COOH)] at 0.02 g/dl or $1.2 \times 10^{-3}N$ in water occurs, in three steps, when the *DP* exceeds some critical value around 100. If a change in the CD is caused by the formation of hydrogen bonds between the peptide groups, we may assign the following mechanism to each step.

As the pH of the solution is lowered from neutrality, the initial nucleation step gradually occurs at from pH 6.5 to 5.5, and a few hydrogen bonds are formed between the peptide groups, either within a single chain or between different chains. The second step is associated with the sharp change in ellipticity at 200 nm at a fixed pH, 5.5, and the midpoint of the transition

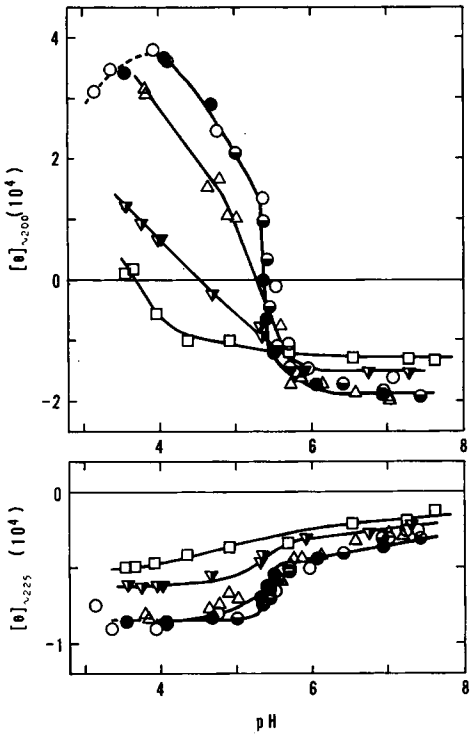

Fig. 5. The pH-induced transition of poly[Cys(CH_2COOH)] of different molecular weights in water (c = 0.02 g/dl): ●, F62-0-A (x = 174); ○, K1201 (x = 140); ◒, F62-0-B (x = 124); △, E602 (x = 63); ▽, E527 (x = 24); □, E515 (x = 11).

is involved in this step at concentrations of 0.02 g/dl. The cooperative step would accompany extensive hydrogen bonding and aggregation of the polypeptide chains, forming the β-conformation substantially. The third, gradual step at lower pH region, down to pH 3.9, may be caused by the rearrangement and further formation of hydrogen bonds, which result in the refolding of the polypeptide chains and a change in the overall shape of the aggregate. The third step might not increase the aggregation number very much but would proceed to complete the structure of β-conformation.

The assignment of the three steps will be supported by the dependence of the transition behavior on the polypeptide concentration and ionic strength, as shown below. If the molecular weight is lower than the critical value, the sharp transition is alleviated and the cooperative step is partially agglomerated with the rearrangement step.

Similar transition steps can be observed in the transition of poly-[Cys($(CH_2)_2COOH$)], as seen in Fig. 4. The three steps of the pH-induced β-coil transition in water would be general, and the presence of the three steps assures that the sample of poly[Cys($(CH_2)_2COOH$)] used here (K917) has a molecular weight higher than the critical value. A lower molecular weight sample (L111) is subject to a less sharp transition at a pH region about 1 pH unit lower.

In comparing the behavior of the CD band at 225 nm with that at 200 nm, we observe that the conformational changes corresponding to the first two steps, i.e., the initiation and cooperative steps, occurring at higher pH are reflected in the change in CD at 225 nm; but the rearrangement and further formation of hydrogen bonds in the last step do not alter the neighboring states of each peptide group appreciably, although changing the overall conformation of polypeptide chains. It seems likely that the n-π^* transition is more perturbed by the short-range interaction between peptide groups, but the π-π^* transition is sensitive to the electronic coupling between peptide groups that are far apart as well as those that are closer.

Figure 6 shows the effect of the polypeptide concentration on the β-coil transition of poly[Cys(CH_2COOH)] in water. The transition pH shifts to a lower value, and the sharp cooperative step becomes more predominant, as the concentration is higher. The latter suggests that the cooperative step is closely related to intermolecular hydrogen bonding or aggregation. Contrarily, the cooperative step will disappear at infinite dilution, and the nucleation step would directly connect with the rearrangement step. Thus, the transition profile at infinite dilution would be similar to that of a polypeptide chain shorter than the critical chain length at a finite concentration. For lower molecular weights, similar sharpening and dominance

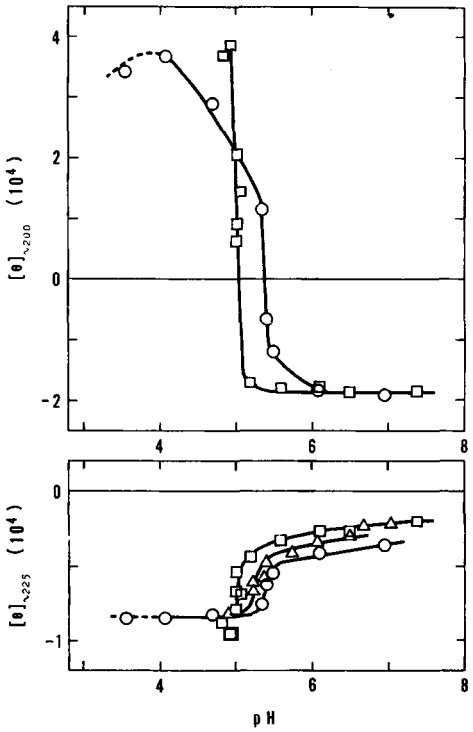

Fig. 6. Effect of polypeptide concentration on the pH-induced transition of poly[Cys-(CH_2COOH)] (F62-0-A) in water: ○, 0.0209 g/dl ($1.31 \times 10^{-3}N$); △, 0.0691 g/dl ($4.29 \times 10^{-3}N$); □, 0.346 g/dl^{-1} ($21.5 \times 10^{-3}N$).

of the cooperative step are observed with increasing polypeptide concentrations. This is illustrated in Fig. 7.

It may be seen that almost equal residue ellipticity at 200 nm is attained before precipitation or gelation occurs, irrespective of the polypeptide concentration. The most perfect β-conformation can be formed in water at any polypeptide concentration. As will be seen below, the effect of polypeptide concentration on the transition pH is parallel to that of ionic strength, and thus it may be considered that the polypeptide concentration plays a role similar to the ionic strength. The destabilization of β-conformation or the lowering of transition pH is not necessarily compatible with intermolecular hydrogen bonding.

Figure 8 shows the effect of added $NaClO_4$ concentration on the transition behavior of poly[Cys(CH_2COOH)]. It is seen that the increase in ionic strength makes the transition sharper and the transition pH lower. By adding $NaClO_4$ to 0.05*M*, the rearrangement step disappears and the initial nucleation step becomes minor. By further addition of $NaClO_4$ to 0.20*M*, the whole transition occurs at a single pH, 4.78, and the nucleation step vanishes.

The addition of salt at constant pH in the transition region destabilizes the β-conformation of poly[Cys(CH_2COOH)] and disrupts it into the ran-

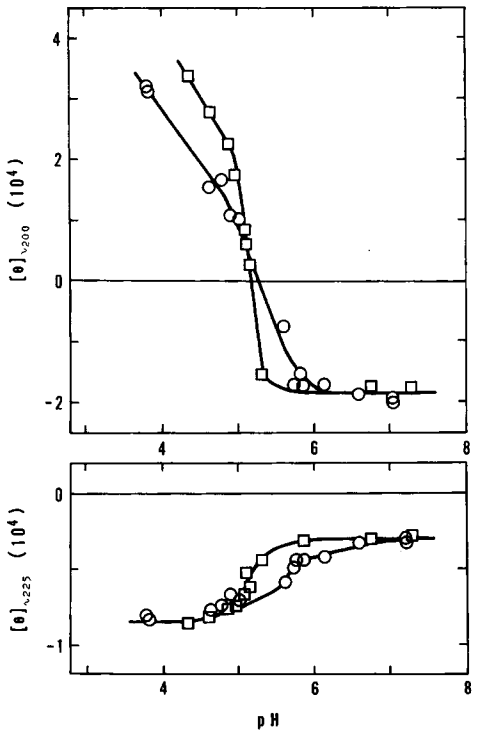

Fig. 7. Effect of polypeptide concentration on the pH-induced transition of poly[Cys(CH_2COOH)] (E602) in water: ○, 0.0195 g/dl ($1.21 \times 10^{-3}N$); □, 0.337 g/dl ($21.0 \times 10^{-3}N$).

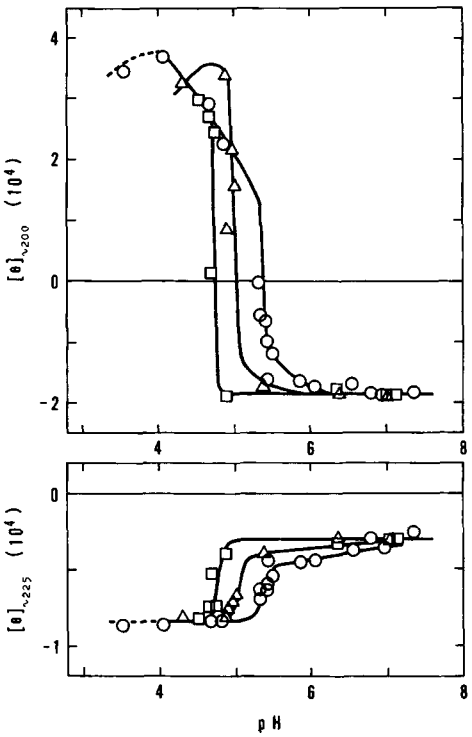

Fig. 8. Effect of $NaClO_4$ concentration on the pH-induced transition of poly[Cys-(CH_2COOH)] (F62-0-A) at 0.0210 g/dl: ○, no salt; △, 0.05*M*; □, 0.20*M*.

dom-coil form. As can be seen from the titration curves,[29] the degree of ionization increases as the salt concentration is increased. Consequently, this observation indicates that the effect of electric repulsion caused by the increased charge density overcomes the electrostatic shielding effect of salt, if $NaClO_4$ exerts no specific effect but only acts like NaCl. Similar destabilization of a hydrogen-bonded conformation by added salt has been found for the α-helix of poly(L-glutamic acid).[37,38]

DISCUSSION

CD of the Random Coil

The CD of the random-coil poly(L-cysteine) derivatives is different from that of random-coil poly(L-glutamic acid) and poly(L-lysine). Specifically, for the n-π^* transition, the former has a negative CD band around 225 nm, whereas the latter has a weak positive band at 220 nm. The difference in CD may be attributed to the effect of the side-chain sulfur atom on the peptide group of poly(L-cysteine) derivatives. The electronic state of peptide groups in the random coil can be reflected in the absorption band for the π-π^* transition. If we compare the values of the residue extinction coefficient at 190 nm for both poly(L-cysteine) derivatives, as given in Table

II, with those for poly(L-glutamic acid) and poly(L-lysine), about 7100,[39,40] we may see the electronic effect of side-chain groups.

Since the electronic effect of the side-chain chromophore on the peptide group should be different for the two poly(L-cysteine) derivatives, owing to the different positions of the carboxyl group, we have different values of residue extinction coefficient for the random coils, 13,000 and 8700. We observe that the CD of the two random-coil polypeptides simply reflects this intrinsic difference in the electronic state of the peptide group, since the dissymmetry factors for the π-π^* transitions are almost equal to each other, as seen in Table IV. Consequently, the modes of electronic coupling of the peptide groups in the two random coils should be almost identical with each other. It seems likely that the perturbation on the n-π^* transition of the peptide groups is also identical in both random coils if we compare the values of rotatory strength given in Table IV.

CD of the β-Conformation

A distinction between the I-β and II-β forms has been made so far, mainly on the basis of the location of the CD band for the n-π^* transition. This may result from different electronic states of peptide groups subject to the effect of the side-chain chromophores, such as the oxygen or sulfur atoms in the II-β form. However, we may further differentiate the β-conformation in solution if the residue ellipticity and the dissymmetry factor for the π-π^* transition are compared for various polypeptides. For example, the values of the dissymmetry factor for the two poly(L-cysteine) derivatives as given in Table IV are different and are much smaller than those for poly(L-lysine)[9] or poly(L-serine)[18]: 3.59 and 6.84, respectively. These differences in CD suggest the formation of β-conformations having different modes of main-chain folding, depending on the nature of the polypeptide species.

It seems natural to suppose that the side-chain interaction plays a more important role in the β-conformation than in the α-helix, so that it is quite reasonable to have different structures of the β-conformation from polypeptide to polypeptide.

Recently, vacuum-uv CD was measured on several Boc-oligopeptides having the β-conformation in the film state.[40] It was found that hepta(L-alanine), hepta(L-valine), and hepta(L-isoleucine) give different ellipticities or different dissymmetry factors over the region from 240 to 140 nm when they are referred to an absorption of unity at the wavelength of maximum absorption. These observations also suggest the presence of different structures of the β-conformation. It should be noticed that α-helical polypeptides give almost identical residue ellipticities down to 170 nm, irrespective of the polypeptide species.[41]

The CD of the β-structure of poly(L-alanine) has been theoretically calculated by Woody,[36] who considers the n-π^* and π-π^* transitions alone. According to him, two criteria are available to distinguish the two types of polypeptide β-structures, parallel chain type and antiparallel chain type.

In the antiparallel chain type, the strongest absorption band should be associated with a weak positive CD band; in the parallel chain type, on the other hand, the major absorption band should be at the same wavelength as a strong negative dichroic band. For the two poly(L-cysteine) derivatives, the absorption band is located at 190 nm, whereas the positive CD band is observed at 200 nm. Clearly, the β-conformation of poly[Cys(CH_2COOH)] and poly[Cys((CH_2)$_2$COOH)] must be of the antiparallel chain type.

Another distinction is related to the difference in wavelength between dichroic and absorption bands. As the number of strands in the β-structure increases, the difference in wavelength decreases from about 16 nm for a single strand to lower values in the following ways: it reaches 5 nm for the antiparallel chain type, but approaches only about 13 nm for the parallel chain type. The difference for both of the poly(L-cysteine) derivatives is about 10 nm, and, hence, the β-conformation must be of the antiparallel chain type.

For the β-structure of antiparallel chain type, we may anticipate a rotatory strength for the n-π^* transition, which is negative in most cases, on the order -0.05 DBM, and sometimes smaller.[36] For the π-π^* transition, we would have a positive rotatory strength around 200 nm, which is on the order of 0.14 DBM.[36] It is seen that the magnitudes of these rotatory strengths are not very sensitive to the number of strands and the *DP* of a chain. Rotatory strengths of the observed dichroic bands were calculated by assuming a Gaussian form of appropriate half-width for the band shape, and their values are given in Table IV. The observed values are comparable with those calculated, especially for poly[Cys((CH_2)$_2$COOH)]. For poly-[Cys(CH_2COOH)] the observed value for π-π^* transition is twice as large.

It should be noticed that Woody's calculation does not take account of possible differences in the electronic state of the peptide groups.

The pH-Induced β-Coil Transition

We have found the presence of a lower limit or a critical length of polypeptide chain for a definite β-coil transition induced by a change of pH. The critical chain length would be dependent on the polypeptide species, and its presence suggests the formation of a definite structure of the β-conformation characteristic of the polypeptide species. In the pH-induced β-coil transition of poly(L-tyrosine), however, the presence of such a critical chain length has never been observed; but two samples of poly(L-tyrosine), which were supposed to have sufficiently high molecular weights, followed different routes or had different transition pH in the β-coil transition when examined by uv absorption.[25]

We have also pointed out the stepwise β-coil transition or the presence of three steps in the pH-induced β-coil transition. This is in contrast to other types of transition such as the helix–coil transition, which is free from aggregation. We have to elucidate the exact nature of the stepwise tran-

sition by means of other methods. Such a stepwise transition can be looked for on the transition curve of poly(L-tyrosine) as well.[25]

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