Effects of Chain Length and Concentration on the β–Coil Conversion of Poly[S-(carboxymethyl)-L-cysteine] in 50 mM NaCl Solutions

Hiroshi Maeda,* Yoshihiro Gatto, and Shoichi Ikeda

Department of Chemistry, Faculty of Science, Nagoya University, Nagoya 464, Japan.

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ABSTRACT: The pH-induced coil-to-β conversion of poly[S-(carboxymethyl)-L-cysteine] (poly[Cys-(CH_2COOH)]) was examined by light scattering and circular dichroism in 50 mM NaCl solutions using five samples of different degrees of polymerization (DP). At a polymer concentration of 1.6×10^{-3} M, the conversion is accompanied by aggregation and is characterized as a microscopic phase separation. The β-structure forms a pseudophase in solution; the transition pH increases with increasing chain length. At 3.2×10^{-4} M, the β-structure is formed by chain folding followed by aggregation. At 1.1×10^{-4} M, the β-structure of the longest chain length ($DP_w = 630$) is formed mostly by chain folding with concomitant weak aggregation; the aggregation number is about 2 at nearly the final stage of the conversion. The coil-to-β conversion at 1.1×10^{-4} M occurs much less steeply than at high concentrations, with a sharpness comparable to that of the helix–coil conversion. Various lines of evidence for the occurrence of the aggregation of folded chains are obtained.

Introduction

An important aspect of the β-structure in solution is the problem of whether it is formed by intermolecular association of extended chains or by chain folding of single polymer molecules. It is likely that these two types coexist in proportion to their relative stabilities; a group of particles consist of folded chains, another group of particles consist of extended chains, and another group of particles are of mixed type consisting of both folded and extended chains.

The intermolecular β-structure formed by the aggregation of extended chains has been reported in the case of short chains or oligopeptides both in organic solvents[1-6] and in aqueous solutions.[7-11] On the other hand, the presence of unaggregated folded-chain β-structure (intramolecular β-structure) has been suggested by several studies,[9,12,13] although measurements of the molecular weight were not carried out in these studies. Kinetic studies are effective in detecting folded chains irrespective of whether their aggregation occurs or not.[14-18] Aggregation of folded chains usually escapes detection unless molecular weight is measured.

Relative stabilities of extended and folded chains in the β-structure depend on the chain length, concentration, and other environmental factors such as pH or ionic strength. Simultaneous measurements of the aggregation number and the content of β-structure will facilitate the characterization of β-structure in solution. A study along this line has been carried out on poly(L-tyrosine) in the concentration range higher than about 200 mg/L.[19] In the present study, the pH-induced coil-to-β conversion of poly[S-(carboxymethyl)-L-cysteine] (poly[Cys-(CH_2COOH)]) is examined by light scattering and circular dichroism (CD) measurements on samples of different chain lengths at different polymer concentrations, including a concentration as low as about 17 mg/L. The ionic strength is kept constant at 50 mM NaCl.

Experimental Section

Five samples of poly[Cys(CH_2COOH)] were synthesized as reported previously.[20] The weight-average molecular weights (M_w) and the degrees of polymerization (DP_w) (in parentheses), determined by light scattering, are as follows: F62-0-A, 10.1×10^5 (630); K1201, 5.8×10^4 (360); F62-0-Al, 4.8×10^4 (300); K1201-1, 3.9×10^4 (240); and E602, 2.6×10^4 (160).

Light scattering was measured on a Chromatix low-angle light scattering photometer KMX-6 at room temperature (24 ± 2 °C) at a wavelength of 633 nm. A 6–7° annulus was chosen to observe the scattered light; this collected light of about 4–5° scattering angle from the solutions. A 0.2-mm field stop was selected in most cases to define the scattering volume. When large aggregates were present, a 0.3-mm field stop was also used to confirm that the results were independent of the size of the field stop. Solutions were filtered through a Millipore membrane filter. When aggregation occurred to a small extent, filters of 0.1-μm pore size were used. Other membrane filters of wider pore sizes (0.22–0.8 μm) were used as the extent of aggregation increased.

Light scattering data were analyzed according to

$$Kc/\Delta R(0) = [mM_0]^{-1} \qquad (1)$$

Here, m, M_0, and c represent respectively the apparent weight-average aggregation number, the weight-average molecular weight of the sample in the unaggregated state, and the polymer concentration (g/mL). The optical constant K is $2\pi^2 n_0^2 (\partial n/\partial c)^2/N_A\lambda^4$, where N_A, λ, and n_0 denote respectively Avogadro's number, the wavelength of the incident light in vacuo (633 nm), and the refractive index of the solvent. The excess light scattering intensity at zero scattering angle $\Delta R(0)$ was approximated by the value observed obtained at a 6–7° annulus.

Refractive index increments $\partial n/\partial c$ at 633 nm were measured on a differential refractometer of the Brice type at 25 ± 0.01 °C on the nondialyzed solutions of a constant NaCl concentration (50 mM) and a constant pH. The aggregation numbers m obtained in the present study are approximate partly because of this approximate procedure. However, this kind of error does not significantly affect the relative changes of the aggregation numbers considered in the present study. The change of the aggregation number accompanying the coil-to-β conversion is followed in the present study. Hence refractive index increments at different values of pH are required. The refractive index increment of the β-structure could not be obtained, however, due to its low solubility. The refractive index increments of random coils were measured at two values of pH 7.0 and 5.05; 0.221 ± 0.009 at pH 7.0 and 0.219 ± 0.009 at pH 5.05 ± 0.04. In the present study, a value of 0.220 is used at all pHs.

CD spectra were taken on a Jasco J-40 A circular dichrograph at 25 ± 0.1 °C, using cells of 1-, 2-, 5-, and 10-mm path lengths. Four scans were accumulated and averaged in most cases.

Solutions of different values of pH were prepared by adding HCl solutions to the fully neutralized solutions. The main part of the CD change occurred within about 3–5 h but a slow and small change followed afterward and was completed within 20 h. Accordingly, measurements were carried out on the solutions about 24 h or longer after their preparation.

The polymer concentration is expressed either in residue molarity C_p (M) or in weight concentration c (g/mL).

Results

CD Spectra at Different Values of pH. The CD spectra of a sample ($DP_w = 630$) in 50 mM NaCl solutions at different values of pH are shown in Figure 1. The polymer concentration is 1.6×10^{-3} M. Spectrum a, ob-

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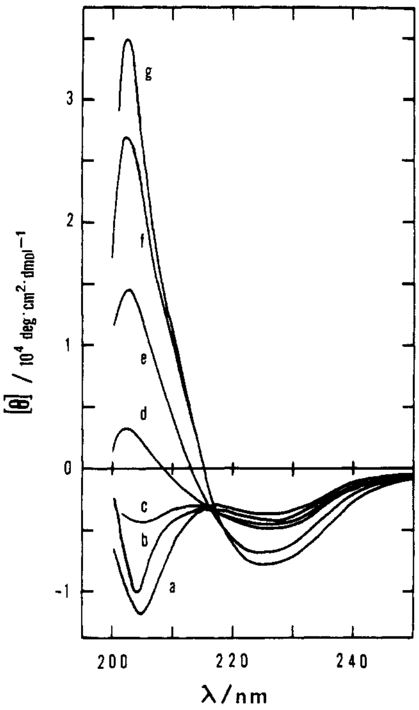

Figure 1. CD spectra of poly[Cys(CH_2COOH)] (DP_w = 630) at different values of pH in 50 mM NaCl solutions. Polymer concentration, 1.6×10^{-3} M. Values of pH: (a) 5.39, (b) 5.10, (c) 5.09, (d) 5.11, (e) 5.03, (f) 4.96, and (g) 4.78.

tained at pH 5.39, shows that the polymers are in the random coil state. As the pH reaches 5.12 ± 0.02, spectra b–d appear. These changes, which are induced by the addition of different amounts of HCl, occur at a nearly constant pH within experimental error. As pH decreases further beyond 5.1, a positive band around 200 nm becomes greater. Thus, the β-structure is formed at pH 5.1 and grows as pH decreases further. The residue ellipticity at 200 nm, $[\theta]_{200}$, is chosen in the present study as a measure of the extent of the conversion between random coils and the β-structure.

As concluded later in the present study, the β-structure at 1.6×10^{-3} M, where the spectra in Figure 1 were taken, is probably an aggregate consisting of both extended and folded chains. On the other hand, the aggregation scarcely occurs at 1.1×10^{-4} M. The CD spectra at 1.1×10^{-4} M and those shown in Figure 1 resemble each other. Accordingly, aggregation does not much distort the CD spectra of the β-structure except in the following respect. The positive band is located in the range 200–202 nm at 1.6×10^{-3} M (Figure 1), whereas it is located in the range 201–204 nm in the spectra at 1.1×10^{-4} M. This difference may represent the difference of the relative population of the folded chains to the extended chains in the β-structure.

pH-Dependent Coil-to-β Conversion at 1.6×10^{-3} M. In Figure 2, the residue ellipticities at 200 and 225 nm, $[\theta]_{200}$ and $[\theta]_{225}$, and the apparent molecular weight (weight average), $\Delta R(0)/Kc$, are given as functions of pH for three samples of different chain lengths. In the case of the sample having DP_w of 630 no significant change of the residue ellipticities occurs until the pH reaches about 5.1, as shown in Figure 2a. At pH 5.1, however, $[\theta]_{200}$ sharply increases or jumps from about -1.3×10^4 to about $+2 \times 10^4$. The value of $[\theta]_{225}$ also jumps at this pH, though the magnitude of the change is small. Thus, the coil-to-β conversion takes place as a kind of phase transition and the transition pH can be defined experimentally. It has been theoretically predicted that the random coil–folded chain conversion takes place as a first-order phase transition in the limit of the infinitely long chain.[21-26] In the present case, however, the aggregation of polymers also occurs at the transition pH, as indicated by the increase in the molecular weight in Figure 2a. Consequently, the observed transition corresponds to a microscopic phase separation rather than the conformational transition predicted for an infinitely long chain. In conclusion, a definite characterization of the β-structure in solution as a pesudophase just as in surfactant micelles is obtained in the present study. This transition-like behavior has been found in the titration involving the β-to-coil conversion,[27] where the transition was assumed to be unimolecular conversion and the corresponding standard free energy change was evaluated from the data.[27] As confirmed in the present study, however, the transition is a kind of phase separation. Accordingly, the previous procedure based on the assumption of unimolecular conversion is misleading.

As pH decreases further beyond the transition pH, $[\theta]_{200}$ increases gradually. The coil-to-β conversion thus occurs

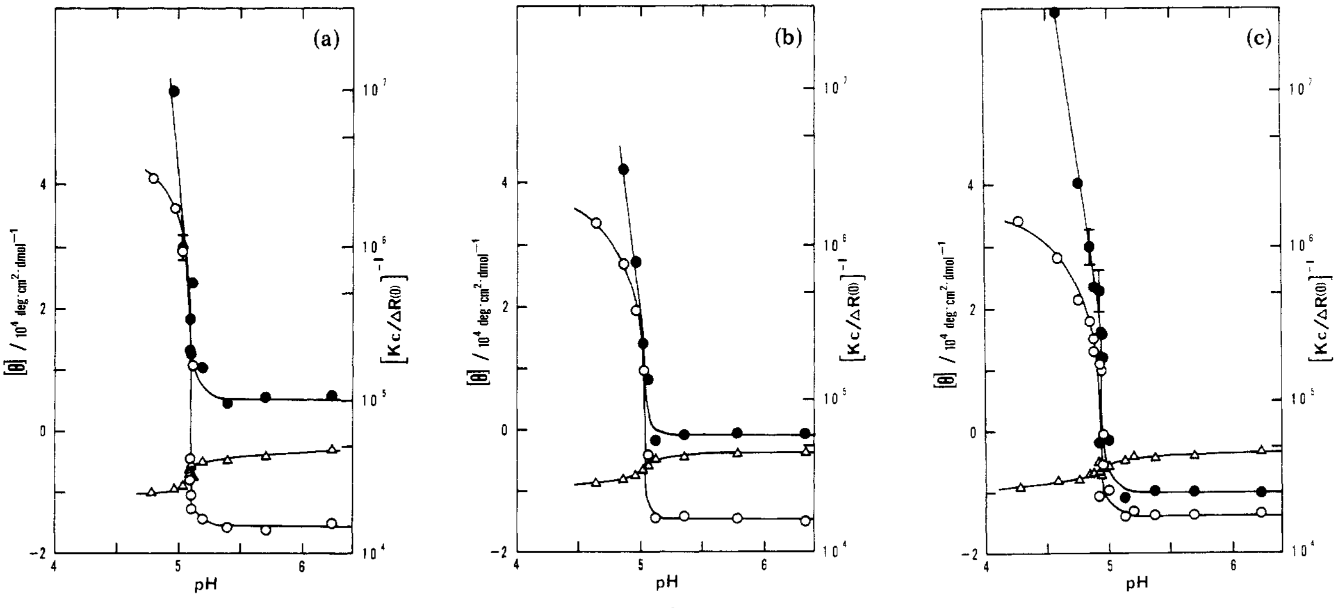

Figure 2. β-coil conversion of poly[Cys(CH_2COOH)] at 1.6×10^{-3} M in 50 mM NaCl solutions: (a) DP_w = 630; (b) DP_w = 360; (c) DP_w = 160. (○) [θ] at 200 nm; (△) [θ] at 225 nm; (●) $\Delta R(0)/Kc$.

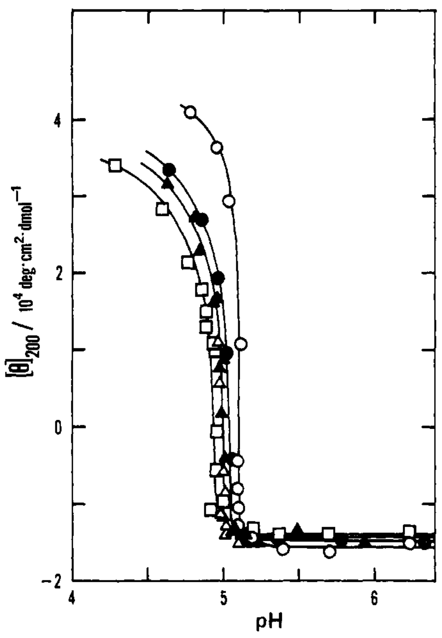

Figure 3. Dependence of residue ellipticities at 200 nm, $[\theta]_{200}$, on pH for five samples at 1.6×10^{-3} M in 50 mM NaCl solutions. DP_w: (○) 630, (●) 360, (△) 300, (▲) 240, and (□) 160.

at least in two steps: the transition step and the posttransition step. At the transition step, the aggregation number increases sharply up to about 10 and then the aggregation develops enormously in the posttransition step, characterized by a logarithmic increase of the aggregation number with a decreasing pH. This logarithmic dependence is most clearly observed in the case of the shortest chain, i.e., Figure 2c.

The above characteristic features of the coil-to-β conversion were commonly found not only for the three samples shown in Figure 2, but also for two other samples (not shown).

In Figure 3, values of $[\theta]_{200}$ are plotted against pH for five samples of different chain lengths. The transition pH, pH_t, can be defined for all samples; it increases with the chain length. The values of pH_t are 5.10, 5.04, 5.00, 5.00, and 4.94 (±0.02) for the samples of DP_w of 630, 360, 300, 240 and 160, respectively. The magnitude of the change of $[\theta]_{200}$ at pH_t is greater the longer the chain. It is about 3.3×10^4 for the largest sample (DP_w = 630), while it is about 1.6×10^4 for the smallest sample (DP_w = 160).

In Figure 4, the results for the coil-to-β conversion are represented by the relation between $[\theta]_{200}$ and the apparent aggregation number *m*. This type of plot has the advantage that it is not distorted by the error associated with pH measurements. Three steps of the coil-to-β conversion can be discriminated more clearly in this type of plot. In the first step, aggregation of random coils occurs, as indicated by a small negligible change of $[\theta]_{200}$ accompanied by an increase of *m*. This implies that nucleation of the β-structure takes place by intermolecular association. This pretransition step is clearly noted in Figure 4 but is not well resolved in Figure 2. The pretransition step does not contribute appreciably to the overall conversion except for the smallest sample (DP_w = 160), for which $[\theta]_{200}$ increases to about -5×10^3 in this step. The transition follows after the pretransition step. In this step, $[\theta]_{200}$ increases sharply at a constant pH accompanying a relatively small increase of *m*. The second step is not unambiguously discriminated in the case of the smallest sample. As the posttransition step, or the third step, the aggregation develops enormously with a small increase of $[\theta]_{200}$. In summary, aggregation of polypeptides occurs in each of the three steps of the coil-to-β conversion at 1.6×10^{-3} M.

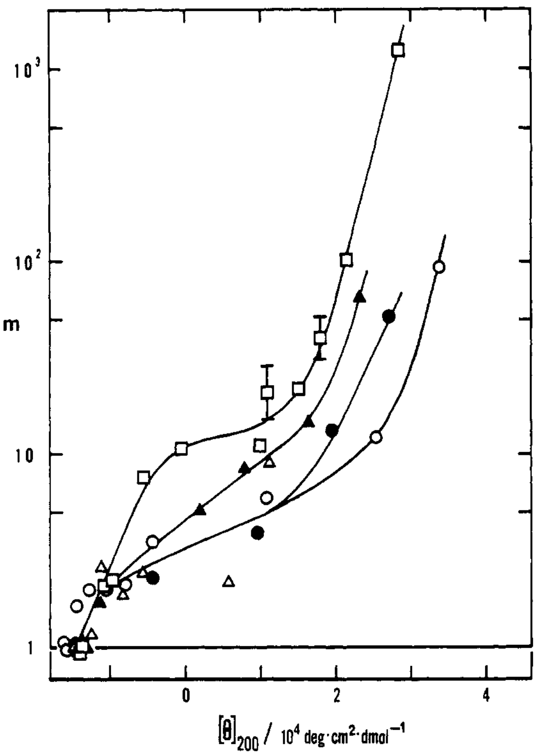

Figure 4. Relation between the apparent aggregation number *m* and $[\theta]_{200}$ at 1.6×10^{-3} M. DP_w: (○) 630, (●) 360, (△) 300, (▲) 240, and (□) 160.

It is expected that the folded-chain β-structure will become less stable as the chain length decreases and that the stabilization of the folded-chain β-structure by aggregation is more effective for shorter chains. Consequently, aggregation of folded chains occurs more extensively for short chains. If, on the contrary, the β-structure is formed by aggregation of the extended chains, then longer chains tend to aggregate predominantly even at the same weight concentration, as found in the study on oligopeptides.[7,8] In Figure 4, the aggregation numbers are generally smaller for longer chains when compared at the same value of $[\theta]_{200}$. This behavior suggests that the aggregation in the present study occurs mainly among folded chains. It is likely, however, that a small amount of extended chains is involved in the aggregates. The extended chains will be associated either with folded chains or among themselves.

Intermolecular association as a nucleation process can be reconciled with the considerable population of folded chains if a mechanism proposed by Hartman et al.[17] plays a central role. According to this mechanism, folding of a chain takes place only when a part of the chain assumes the β-conformation by association with another chain.

pH-Dependent Coil-to-β Conversion at 3.2×10^{-4} M. In Figure 5, the coil-to-β conversion of the highest molecular weight sample (DP_w = 630) obtained at 3.2×10^{-4} M is given. Although $[\theta]_{200}$ changes sharply, the change is continuous without any significant jump. The transition-like nature is largely obscured and the transition pH cannot be defined at this low concentration. Instead, the conversion is characterized in terms of the pH at the midpoint of the conversion. The conversion begins to occur at about pH 5.1 and the midpoint of the conversion is located at approximately pH 5.0. This value is lower than the transition pH at 1.6×10^{-3} M by about 0.1 unit. The conversion at 3.2×10^{-4} M was also examined for the lowest molecular weight sample (DP_w = 160) (not shown).

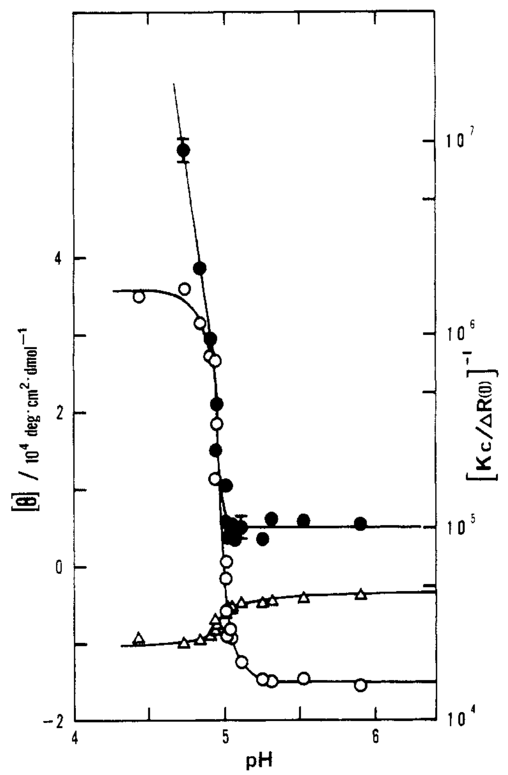

Figure 5. β-coil conversion of poly[Cys(CH$_2$COOH)] (DP$_w$ = 630) at 3.2 × 10^{-4} M in 50 mM NaCl solutions. (○) $[\theta]_{200}$, (△) $[\theta]_{225}$, and (●) $\Delta R(0)/Kc$.

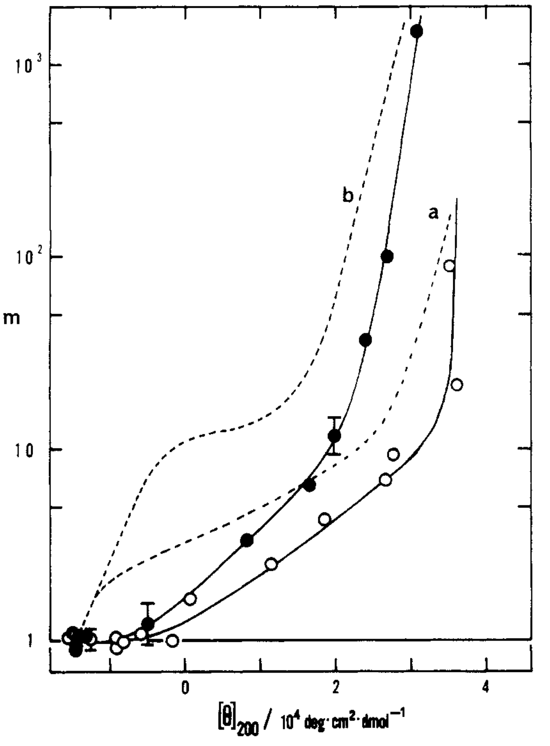

Figure 6. Relation between the apparent aggregation number m and $[\theta]_{200}$ at 3.4 × 10^{-4} M. DP$_w$: (○) 630 and (●) 160. Dashed curves a and b represent the results at 1.6 × 10^{-3} M on the samples of DP$_w$ = 630 and 160, respectively.

However, the difference between the conversion pH and the transition pH at 1.6 × 10^{-3} M could not be detected in this sample, due probably to the polydispersity of the sample.

In Figure 6, the results at 3.2 × 10^{-4} M are given in the same plot as in Figure 4 for the two samples DP$_w$ = 630 and 160. The effects of polymer concentration are best examined by comparing Figure 6 with Figure 4. To facilitate the comparison, the results at 1.6 × 10^{-3} M are again shown by dashed curves. The coil-to-β conversion at 3.2 × 10^{-4} M takes place in three steps for both samples. However, these steps are different from those at 1.6 × 10^{-3} M, except for the third step. An important difference is found at the first step of the conversion. The nucleation step by the intermolecular association disappears at 3.2 × 10^{-4} M, in constrast with the results at 1.6 × 10^{-3} M. Instead, the β-structure is formed entirely by intramolecular chain folding at this low concentration. Further, a unimolecular conversion without aggregation takes place in the range where $[\theta]_{200}$ increases along the line m = 1 in Figure 6, although the β-content is very small in this range. Then the growth of the β-structure follows this intramolecular chain-folding process, accompanying a small extent of aggregation. This growth step is characterized by an approximately linear relation between $[\theta]_{200}$ and m. It is likely that in this step the aggregation of the folded chains is predominant over the aggregation of the extended chains. The third step is very similar to the posttransition step at 1.6 × 10^{-3} M; extensive aggregation occurs with a slight or negligible increase of $[\theta]_{200}$. It is to be noted in Figure 6 that even a sample of DP$_w$ = 160 forms the β-structure by chain folding, since the intermolecular nucleation also disappears for this sample.

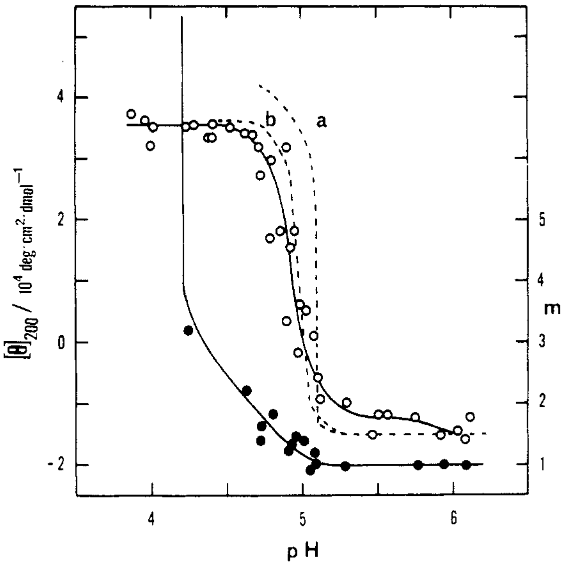

Figure 7. β-coil conversion of poly[Cys(CH$_2$COOH)] (DP$_w$ = 630) at 1.1 × 10^{-4} M in 50 mM NaCl solutions. (○) $[\theta]_{200}$ and (●) the apparent aggregation number m. Dashed curves a and b represent the values of $[\theta]_{200}$ obtained at 1.6 × 10^{-3} and 3.2 × 10^{-4} M, respectively.

pH-Dependent β-Coil Conversion at 1.1 × 10^{-4} M. From the data given in the preceding sections, it is advantageous to perform the coil-to-β conversion at a concentration as low as possible to substantiate the presence of the folded-chain β-structure without aggregation. The measurements of CD and light scattering were carried out at 1.1 × 10^{-4} M on the highest molecular weight sample (DP$_w$ = 630). At this concentration, the scattered light intensity of the solution containing no aggregates is about 1.4 times that of the solvent. The results are shown in Figure 7. In Figure 7, the values of $[\theta]_{200}$ at other two concentrations are again indicated by dashed curves for the sake of comparison. In Figure 7, m is given on a linear scale instead of the logarithmic scale in Figures 2, 4, 5, and 6.

The coil-to-β conversion at 1.1 × 10^{-4} M is qualitatively similar to that found at 3.2 × 10^{-4} M. However, the ag-

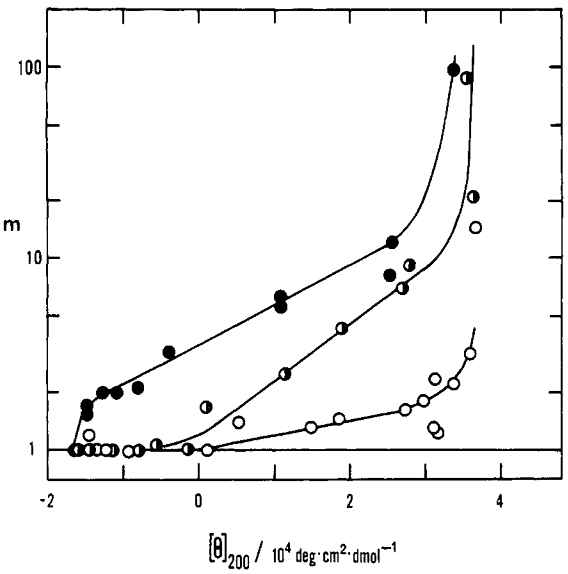

Figure 8. Relation between the apparent aggregation number m and $[\theta]_{200}$ of poly[Cys(CH_2COOH)] (DP_w = 630) at different polymer concentrations: (○) 1.1×10^{-4} M; (◑) 3.2×10^{-4} M; (●) 1.6×10^{-3} M.

gregation is even more suppressed at 1.1×10^{-4} M than at 3.2×10^{-4} M. The apparent aggregation number is only 2 at the pH range where the conversion is almost completed, as shown in Figure 7. Consequently, the essential part of the conversion at 1.1×10^{-4} M occurs as a unimolecular transconformation between a folded-chain β-structure and a random coil, with concomitant aggregation of the folded chains. In Figure 7, the values of $[\theta]_{200}$ in random coil region (pH >5.1) are not consistent between 1.1×10^{-4} M and the other two concentrations. A value of about -1.2×10^4 was often obtained at 1.1×10^{-4} M, while a value of about -1.5×10^4 was obtained at two other concentrations. The reason for these inconsistent results is not clear at present. In spite of this inconsistency, the pH range where the β-structure begins to form is common to the three concentrations, i.e., about 5.1. The formation of the β-structure becomes more gradual as the polymer concentration decreases. It is clearly understood that the sharpening or cooperative nature of the conversion at high concentrations stems from aggregation.

A novel feature of the data at 1.1×10^{-4} M is that a constant level of $[\theta]_{200}$ is attained in the low-pH region. This is the first example in the coil-to-β conversion in aqueous media. The values of $[\theta]_{200}$ can be uniquely assigned to the two conformations in the 1.1×10^{-4} M case. It is 3.55×10^4 for the folded-chain β-structure without aggregation and -1.20×10^4 for random coils.

The values of $[\theta]_{200}$ in the low-pH region are larger for the highest concentration (1.6×10^{-3} M) than the level-off value at low concentrations. This indicates that the aggregation contributes to the optical activity and/or increases the fraction of the β-structure. It is likely that this contribution arises from the coexistence of the extended chains involved in the aggregation process at high concentrations.

At 1.1×10^{-4} M, a constant value of $[\theta]_{200}$ is obtained in spite of the presence of the aggregates. Accordingly, the aggregation at 1.1×10^{-4} M occurs exclusively among folded chains.

In Figure 8, the apparent aggregation numbers m are plotted against $[\theta]_{200}$ for the largest sample (DP_w = 630) at three concentrations. The effects of the concentration are manifest. At 1.1×10^{-4} M, the aggregation is largely suppressed. Nevertheless, deviation from the line m = 1 occurs at a value of $[\theta]_{200}$, which is still not large enough to describe the polymer as β-structure without aggregation.

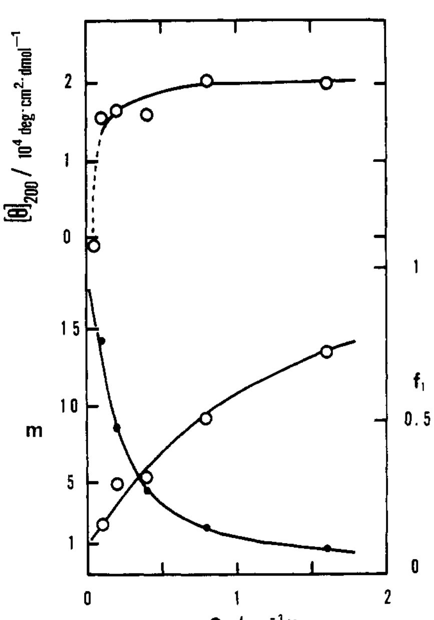

Figure 9. Effects of polymer concentration C_p on $[\theta]_{200}$ and the apparent aggregation number m at a constant pH. Sample: DP_w = 360. pH 4.90 ± 0.05. The weight fraction of unaggregated polymers calculated by eq 2, f_1, is denoted by filled circles.

Effect of the Polypeptide Concentration (C_p) at a Fixed pH. In the present study, various lines of evidence are presented indicating that the β-structure is formed by intramolecular chain folding under a variety of conditions. At high concentrations, the β-structure is characterized as aggregates forming a pseudophase. These aggregates consist of both folded and extended chains. At low concentrations, on the other hand, the intramolecular nucleation is the only process to form the β-structure. It is likely that the aggregation at low concentrations occurs among folded chains.

In the present section, another line of evidence is provided indicating the aggregation of the folded chains by examining the effect of the concentration on the coil-to-β conversion of a sample (DP_w = 360) at a fixed pH of about 4.9. In Figure 9, both $[\theta]_{200}$ and m are given in the concentration range lower than about 1.6×10^{-3} M. The residue ellipticity changes only slightly with C_p except for the lowest one. The aggregation number, on the other hand, increases considerably with C_p. This type of change, the aggregation with a small change of $[\theta]_{200}$, resembles the third step of the β-coil conversion at high concentrations. However, the constant levels of $[\theta]_{200}$ are different between these two situations. It is about 2.0×10^4 in Figure 9, while it is about $(3–4) \times 10^4$ encountered in the third step. The data of Figure 9 represent the concentration dependence of the second step and hence provide a novel feature. Aside from the data at the lowest concentration, the aggregation occurs without a significant accompanying change of $[\theta]_{200}$. Such aggregation cannot occur for extended chains. Consequently, the aggregation must be occurring among the folded-chain β-structures.

If ideal solutions of aggregates are assumed, the weight fraction of monomer f_1 can be evaluated from the data on the weight-average aggregation number m.[28]

$$\ln f_1 = \int_0^{C_p} [m^{-1} - 1]\left(\frac{1}{C_p}\right) \mathrm{d}C_p \qquad (2)$$

The obtained values of f_1 are also shown in Figure 9. When

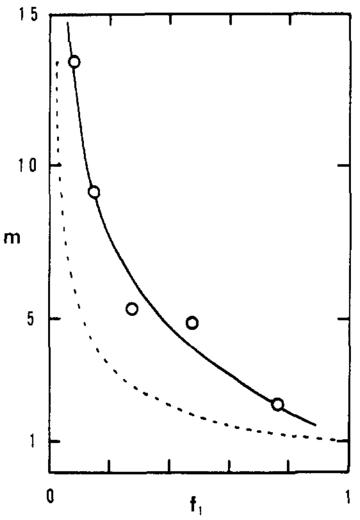

Figure 10. Relation between the apparent aggregation number m and the weight fraction of unaggregated polymer f_1. Data are taken from Figure 9. Dashed line represents the expected results for isodesmic association: $m + 1 = 2/f_1^{1/2}$.[29]

m is plotted against f_1 (a standard plot[29]) in Figure 10, the data resemble the dashed curve corresponding to the isodesmic association case, although a definite and significant deviation from it is apparent. The deviation indicates that the aggregation occurs more extensively and/or the weight fraction of monomer f_1 is larger than that expected from isodesmic association. It is most likely that the deviation can be ascribed to the "contamination" of aggregates of extended chains. These aggregates are resistant against moderate dilution but dissociate eventually at extremely low concentrations. A sharp decrease of $[\theta]_{200}$ at 5×10^{-5} M is probably related to the dissociation of the aggregates of extended chains which are too short to fold to the β-structure.

Discussion

Comparison with the Helix–Coil Conversion. Based on the data at 1.1×10^{-4} M, the fraction of residues in the β-structure, f_β, can be unambiguously calculated from the CD data given in Figure 7. In Figure 11, values of f_β at 1.1×10^{-4} M are given as a function of pH. The conversion curve can be approximated as representing a unimolecular conversion. Thus, the conversion curve can be compared with the helix–coil conversion, which is another unimolecular conversion. The data on the helix–coil conversion of poly(L-glutamic acid) (PGA) on a sample of $DP_w = 450$ in 0.2 M NaCl at a polymer concentration of 1.88×10^{-2} M are taken from the work of Nagasawa and Holtzer[30] and are also shown in Figure 11. The width of the conversion is very similar in the two cases: about 1.0 pH unit for the present β–coil conversion and about 0.9 pH unit for the helix–coil conversion of PGA. Sharp transition-like behavior has often been observed in the β-to-coil conversion, simply because the working concentrations are usually much higher than 1×10^{-4} M. The pH value at the midpoint of the conversion, pH_m, is 4.92 for the β-to-coil conversion, while it is 5.12 at 0.2 M NaCl but 5.70 at 0.005 M NaCl for the helix–coil conversion of PGA.[30] At the midpoint pH, degrees of ionization α and electrostatic free energies G^{el} (per residue) are different for the two conformations in equilibrium, β-structure and coil (or α-helix and coil). When these differences are denoted as $\Delta\alpha_{c\to\beta}$ and $\Delta G^{el}{}_{c\to\beta}$ (or $\Delta\alpha_{c\to\alpha}$ and $\Delta G^{el}{}_{c\to\alpha}$), the free energy difference of the fully protonated polypeptides between the two conformations $\Delta G°_{c\to\beta}$ (or $\Delta G°_{c\to\alpha}$) can be expressed as follows based on the all-or-none assumption. ($\Delta G°$ is negative.)

$$-\Delta G° = \Delta G^{el} + 2.303RT(-\Delta\alpha)(pH_m - pK_0) \qquad (3)$$

The second term on the right-hand side of eq 3 is positive, while the first term ΔG^{el} can be positive or negative. Generally, $\Delta\alpha_{c\to\beta}$ is more negative than $\Delta\alpha_{c\to\alpha}$. The intrinsic dissociation constants K_0 are considerably different for these two carboxyl groups: $pK_0 = 3.25$ for poly[Cys(CH$_2$COOH)][31] and $pK_0 = 4.45$ for PGA.[30] For the β-structure, $pH_m - pK_0 = 1.67$ (50 mM NaCl), while $pH_m - pK_0 = 0.67$ (0.2 M NaCl) or 1.25 (5 mM NaCl) for the α-helix of PGA.[30] Accordingly, the stabilization due to the second term on the right-hand side of eq 3 is greater for the β-structure of poly[Cys(CH$_2$COOH)] than for the α-helix of PGA, since $(pH_m - pK_0)$ is greater for the former than the latter if compared at the same ionic strength.

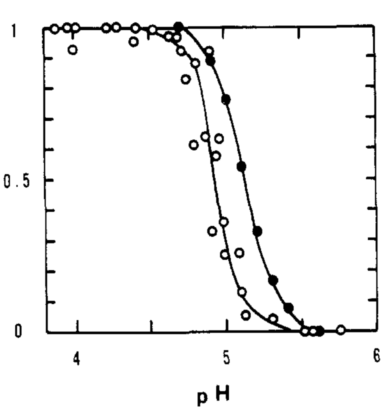

Figure 11. β–coil conversion of pol][Cys(CH$_2$COOH)] ($DP_w = 630$) and helix–coil conversion of poly(L-glutamic acid) ($DP_w = 450$) in 50 mM NaCl solutions. f_β (○) and f_h (●) denote the fractions of residues in the β-structure and the α-helix, respectively. Polymer concentration: 1.1×10^{-4} M (○) and 1.88×10^{-2} M (●). Data on poly(L-glutamic acid) are taken from ref 30.

Free Energy Change of the Coil-to-β Conversion. The standard free energy change $\Delta G°$ appearing on the left-hand side of eq 3 can be evaluated from the potentiometric titration at a low concentration where the conversion occurs unimolecularly,[30,32,33] although it is not feasible to carry out ordinary potentiometric titrations at a low concentration of 1.1×10^{-4} M with due accuracy. A highly approximate evaluation of the titration curve can be made, however, when previous potentiometric titration data on poly[Cys(CH$_2$COOH)][31] are combined with the present results, as shown below.

Potentiometric titration data of a sample K1201 ($DP_w = 360$) at 9.6×10^{-3} M in 50 mM NaCl solutions are taken from the previous study[31] and are given in Figure 12. Since the titration curve a of random coils hardly depends on the chain length and the polymer concentration, it can be used for the sample (DP = 630) at 1.1×10^{-4} M. On the other hand, the titration curve b, calculated for an infinitely large plane (Gouy model[34]), is not applicable to the sample ($DP_w = 630$) at 1.1×10^{-4} M. Filled and open circles represent the experimental data. [The previous study[31] should be consulted for a discussion of the difference between the two circles.] Various lines representing constant values of pH are also shown in Figure 12. The experimental titration data show that the β-to-coil transition occurs at a constant pH of 5.04. This is in excellent agreement with the transition pH of the same sample at 1.6×10^{-3} M found in the present study ($pH_t = 5.04$). This agreement indicates that the titration curves are identical at the two concentrations, 1.6×10^{-3} and 9.6×10^{-3} M. For the higher side chain homologue poly[S-

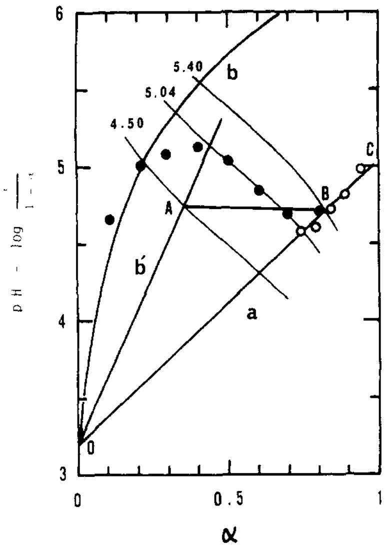

Figure 12. Approximately evaluated titration curve of poly[Cys(CH$_2$COOH)] (DP$_w$ = 630) at 1.1×10^{-4} M in 50 mM NaCl solutions. The degree of ionization is denoted as α. Filled and open circles are experimental data on the sample DP$_w$ = 360. Line a represents the titration curve of random coils. Curve b represents the calculated titration curve for an infinitely large plane. Line b′ represents the assumed titration curve for the unaggregated pleated sheet formed by the sample DP$_w$ = 630. Curves representing constant values of pH are drawn for pH 4.50, 5.04, and 5.40. Experimental data and curve b are taken from ref 31.

(carboxyethyl)-L-cysteine], it has been shown that the titration curves in 0.2 M NaCl are independent of the polymer concentration in the range between 0.89×10^{-3} and 2×10^{-2} M.[31] Since the titration data of Figure 12 are obtained at 9.6×10^{-3} M, it is expected that the aggregation should occur in the transition step and the stacking of the pleated sheets should occur at the posttransition step, when the present results are taken into consideration. Accordingly, the titration curve of a pleated sheet (without stacking) should pass through a point on the experimental titration curve in the range where it coincides with the line pH = 5.04. In order to evaluate the upper limit for $-\Delta G°$, a straight line b′ is drawn through the point where experimental titration data begin to deviate from the line pH = 5.04. This line b′ is tentatively used here as the titration curve of the β-structure of a sample (DP$_w$ = 630) at 1.1×10^{-4} M. From Figure 11, the coil-to-β conversion occurs in the pH range between 4.50 and 5.40. As an approximation, the titration curve in the conversion region at 1.1×10^{-4} M is given by a straight line connecting the two titration curves a and b′ at respective points of detachment (A and B). Although the procedure looks like an oversimplification, it turns out to be pertinent, since it approximately represents the conversion at a constant surface potential, which has been found in the helix–coil conversion of PGA.[30,35–37]

In this way, a hypothetical titration curve (OABC) of a sample (DP$_w$ = 630) at 1.1×10^{-4} M is obtained based on the previous titration data combined with the present results. The area OABO yields 500 cal/mol for $-\Delta G°$. The correct titration curve for an unaggregated pleated sheet formed by a sample (DP$_w$ = 630) has undoubtedly lower surface potentials than those given by line b′. Also, the surface potential at point A should be lower than that at point B, in order for the line AB to have a positive slope. All these considerations indicate that the estimated value of 500 cal/mol should be regarded as the upper limit for $-\Delta G°$.

Extensive Aggregation Step. In the present coil-to-β conversion, a common final step is found at two polymer concentrations, 1.6×10^{-3} and 3.2×10^{-4} M, irrespective of the molecular weights of the samples. This step is referred as the posttransition step at 1.6×10^{-3} M. This step is characterized by an aggregation process accompanying a small increase of the β-content. Such an aggregation step is most likely the stacking of the pleated sheets.

The disappearance of an isodichroic point has been used as a diagnostic sign of the stacking of the pleated sheets.[10,38] In the present study, the isodichroic point at 218 nm disappeared in the case of the lowest molecular weight sample (DP$_w$ = 160) as the extensive aggregation step occurred (not shown). This result supports the interpretation of the extensive aggregation step as the stacking of the pleated sheets. At the same time, it provides another support for the validity of the proposed diagnostic tool. It is to be noted that the molecular weight measurements cannot distinguish the stacking process from lateral association of the pleated sheets through hydrogen bonds between peripheral peptide groups.

A prominent feature of the third step is noticed in Figures 4 and 6. There is a threshold value of the aggregation number m required for the third step to occur. This value lies between 10 and 20. It is interesting to note that there is no threshold for $[\theta]_{200}$, which is proportional to the β-content. The value of $[\theta]_{200}$ at the beginning of the third step is smaller for smaller chains. It also depends on the concentration. In the case of the smallest sample (DP$_w$ = 160), for example, extensive aggregation takes place in the range $[\theta]_{200} \gtrsim 1.5 \times 10^4$ at 1.6×10^{-3} M, while at 3.2×10^{-4} M it occurs in the range $[\theta]_{200} \gtrsim 2 \times 10^4$.

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Registry No. Poly[Cys(CH$_2$COOH)] (homopolymer), 29433-95-2; poly[Cys(CH$_2$COOH)] (SRU), 31851-29-3; NaCl, 7647-14-5.

References and Notes

(1) Goodman, M.; Scmitt, E. E. *J. Am. Chem. Soc.* **1959**, *81*, 5507.
(2) Goodman, M; Schmitt, E. E.; Yphantis, D. A. *J. Am. Chem. Soc.* **1962**, *84*, 1288.
(3) Toniolo, C.; Bonora, G. M. "Peptides: Chemistry, Structure, and Biology"; Walter, R., Meienhofer, J., eds.; Ann Arbor Science Publishers: Ann Arbor, MI, 1975; p 145.
(4) Okahashi, K.; Ikeda, S. *Biopolymers* **1979**, *18*, 2105.
(5) Ikeda, S.; Okahashi, K. *Biopolymers* **1979**, *18*, 2115.
(6) Imae, T.; Okahashi, K.; Ikeda, S. *Biopolymers* **1981**, *20*, 2553.
(7) Rinaudo, M.; Domard, A. *J. Am. Chem. Soc.* **1976**, *98*, 6360.
(8) Maeda, H.; Iwase, T.; Ikeda, S. *Polym. J.* **1984**, *16*, 471.
(9) Maeda, H.; Kadono, K.; Ikeda, S. *Macromolecules* **1982**, *15*, 822.
(10) Saito, K.; Maeda, H.; Ikeda, S. *Biophys. Chem.* **1982**, *16*, 67.
(11) Maeda, H.; Saito, K.; Ikeda, S. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 602.
(12) Patton, E.; Auer, H. E. *Biopolymers* **1975**, *14*, 849.
(13) McKnight, R. P.; Auer, H. E. *Macromolecules* **1976**, *9*, 939.
(14) Davidson, B.; Fasman, G. D. *Biochemistry* **1967**, *6*, 1616.
(15) Wooley, S.-Y. C.; Holzwarth, G. *Biochemistry* **1970**, *9*, 3604.
(16) Snell, C. R.; Fasman, G. D. *Biochemistry* **1973**, *12*, 1017.
(17) Hartman, R.; Schwaner, R. C.; Hermans, J., Jr. *J. Mol. Biol.* **1974**, *90*, 415.
(18) Yamamoto, H.; Yang, J. T. *Biopolymers* **1974**, *13*, 1109.
(19) Senior, M. B.; Gorrell, S. L. H.; Hamori, E. *Biopolymers* **1971**, *10*, 2387.
(20) Ikeda, S. *Biopolymers* **1967**, *5*, 359.
(21) Zwanzig, R.; Lauritzen, I. *J. Chem. Phys.* **1968**, *48*, 3351.
(22) Birshtein, T. M.; Elyashevich, A. M.; Skvortsov, A. M. *Mol. Biol. (Engl. Transl.)* **1971**, *5*, 78.
(23) Kano, F. *J. Phys. Soc. Jpn.* **1976**, *41*, 219.
(24) Adonts, V. G.; Birshtein, T. M.; Elyashevich, A. M.; Skvortsov, A. M. *Biopolymers* **1976**, *15*, 1037.
(25) Wako, H.; Saito, N. *J. Phys. Soc. Jpn.* **1978**, *44*, 1931.
(26) Wakana, H.; Shigaki, T.; Saito, N. *Biophys. Chem.* **1982**, *16*, 275.
(27) Pederson, D.; Gabriel, D.; Hermans, J., Jr. *Biopolymers* **1971**, *10*, 2133.

(28) Steiner, R. F. *Arch. Biochem. Biophys.* **1952**, *39*, 333.
(29) Chun, P. W.; Kim, S. J. *Biochemistry* **1970**, *9*, 1957.
(30) Nagasawa, M.; Holtzer, A. *J. Am. Chem. Soc.* **1964**, *86*, 538.
(31) Maeda, H.; Ikeda, S. *Biopolymers* **1975**, *14*, 1623.
(32) Zimm, B. H.; Rice, S. A. *Mol. Phys.* **1960**, *3*, 391.
(33) Leyte, J. C.; Mandel, M. *J. Polym. Sci., Part A* **1964**, *2*, 1879.
(34) Verwey, E. J. W.; Overbeek, J. Th. G. "Theory of the Stability of Lyophobic Colloids"; Elsevier: New York, 1948; p 22.
(35) Wada, A. *Mol. Phys.* **1960**, *3*, 409.
(36) Olander, D. S.; Holtzer, A. *J. Am. Chem. Soc.* **1968**, *90*, 4549.
(37) Hermans, J., Jr. *J. Phys. Chem.* **1966**, *70*, 510.
(38) Maeda, H.; Ooi, K. *Biopolymers* **1981**, *20*, 1549.

Efficient Dynamical Monte Carlo Method for Dense Polymer Systems

Hagai Meirovitch

Polymer Research Department, The Weizmann Institute of Science, Rehovot 76100, Israel.

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ABSTRACT: A new dynamical Monte Carlo (MC) procedure for chains with excluded volume (EV) is suggested and applied to single-chain models on a simple cubic lattice. In each MC step of our procedure we select at random three neighbor "beads" and calculate for them the entire group of *allowed* local conformations (ALCs); an ALC is a conformational change of the three beads, which does not violate the EV condition. A new ALC is then randomly selected out of the group of ALCs. This new procedure differs from the conventional one (e.g., Verdier and Stockmayer), in which a trial local conformation (not necessarily an *allowed* one) is first selected and becomes the new ALC only if the EV condition is satisfied. For a single chain without EV our method leads to the expected Rouse-like dynamics, i.e., $\tau \sim N^2$ and $D \sim N^{-1}$, where τ, N, and D are the longest relaxation time, the number of beads, and the diffusion coefficient, respectively. For chains with EV we obtain $\tau \sim N^{2+\epsilon}$, where ϵ is close to the theoretical value 0.2. The use of our method for dense polymer systems is expected to be more efficient than the conventional one where most of the trial local conformations will be forbidden. To demonstrate this, the two methods have been applied to a single chain on a lattice which is partially filled with "obstacles" (i.e., a lattice site with an obstacle is also excluded for the chain). Indeed, our method has always led to significantly larger values of D than the conventional method and has been found to be significantly more efficient.

1. Introduction

The dynamics of polymer chain models with excluded volume (EV) is a difficult analytical problem and has therefore been studied mainly with computer simulation.[1-12] To make the simulation of long chains tractable, highly simplified models have been employed, in which the chain is represented by N connected nonintersecting "beads" constrained to lie on a lattice.[1-12] These models have been conveniently simulated with the Monte Carlo (MC) method using various types of bead movement.[1-12] In all of these studies the repeated MC step consists of the following two stages: (1) a small group of beads is selected at random; (2) a trial conformational change of these beads (which we call here a local conformation) is randomly selected and the EV condition is checked; if it is not violated the trial local conformation is accepted and the beads are moved to their new positions. In the other case, no conformational change is performed. This procedure has been found to be efficient for single-chain models in which the EV effect is relatively weak. However, it is expected to be highly inefficient for dense, many-chain systems where most of the trial local conformations will be forbidden. In this article we, therefore, suggest modifying the "blind" selection of local conformations (described in stage 2) by first calculating the entire group of *allowed* local conformations (ALCs), i.e., those which do not violate the EV restriction, and then selecting at random from this group a new ALC. In what follows the new method is described in detail and tested on single-chain models with and without EV on a simple cubic lattice. We are mainly interested in the dependence of the longest relaxation time, τ, on chain length, where τ is extracted from the autocorrelation functions of global properties of the chain, such as the end-to-end distance and the radius of gyration.

For a chain without EV, τ is expected to behave like in the Rouse model,[1,3,6,11,12,17-19] i.e.

$$\tau \sim N^2 \tag{1}$$

However, when the EV interaction is taken into account, one would expect the relaxation to slow down

$$\tau \sim N^{2+\epsilon} \tag{2}$$

de Gennes[20] has predicted (on the basis of phenomenological scaling arguments) $\epsilon = 0.2$ in three dimensions, which is consistent with ϵ expansion obtained by Jasnow and Moore.[21] It should be pointed out that this result has been derived for the Rouse model[17] but one would expect it to be universal, i.e., independent of the details of the model and of the particular mechanism of relaxation used.[11,13-15,23] However, MC studies carried out by Verdier's group[1-6] have all led to $\epsilon \sim 1$. Hilhorst and Deutch[22,23] have argued that this unexpected strong slowing down is due to the lattice restriction and can be removed by using, in addition to Verdier's one-bead flip, also a 90° crankshaft motion. Indeed, simulations based on these types of motion have led to values of ϵ close to 0.2.[8-12] Since our method also includes these bead movements, we expect it to also lead to the expected value $\epsilon = 0.2$. It should be pointed out that $\epsilon \sim 0.2$ has also been obtained for a nonlattice model by Ceperley et al.[14] On the other hand, a molecular dynamics study of Bishop et al [13] yielded $\epsilon \sim -1$ but for very short chains.

We compare the efficiency of our MC method with that of the conventional one, which is based on a one-bead flip and a 90° crankshaft motion. In order to demonstrate the advantage of using the new method for dense, many-chain systems, we are also studying by both methods a similar model, of a single chain with EV moving on a lattice, which

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