β Structure of Poly[S-(carboxymethyl)-L-cysteine] in Aqueous Solutions by Intermolecular Association and Intramolecular Chain Folding

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ABSTRACT: The β structure of poly[S-(carboxymethyl)-L-cysteine] is formed by intermolecular association in a low molecular weight sample, while intramolecular chain folding is suggested for the β structure of a high molecular weight sample. The β–coil change induced by a change of either charge density or polymer concentration was monitored by CD in media containing little or no added salt. Although the β structure of the low molecular weight sample was not formed from random coils at a low concentration (8×10^{-5} N), it was formed at a high concentration (4×10^{-3} N). The β structure, formed at 4×10^{-3} N, remained stable when diluted to 8×10^{-5} N. The association–dissociation was thus irreversible at 8×10^{-5} N, while it was reversible at 4×10^{-3} N. For the high molecular weight sample, reversibility of the β–coil transition was complete at 3×10^{-3} N but incomplete at 3×10^{-5} N. In a medium of excess salt (19 mM $NaClO_4$) and a constant pH (4.5), the residue ellipticity at 200 nm showed a transition from 2.4×10^4 to 4.0×10^4 deg cm^2 dmol^{-1} in the range $(0.5–1) \times 10^{-4}$ N. An intramolecular β structure without aggregation is suggested for a range below 5×10^{-5} N, and the transition is ascribed to aggregation. For both intermolecular and intramolecular β structures, the aggregates could not be effectively dissociated by dilution.

An important aspect of the β structure of polypeptides in solution is the problem of whether it is formed by intermolecular association or by chain folding of a single polymer molecule. It is likely that these two types coexist in proportion to their relative stabilities and that other mixed types are also present. Accordingly, assignment of either of the two types can be done only under favorable conditions. Since a certain chain length will be required for chain folding to occur, chain length will be one of the important factors in determining relative stability of either the intramolecular or the intermolecular process. For this reason, studies of samples of a broad molecular weight distribution will prevent us from obtaining unambiguous results.

The problem of inter- and intramolecular β structure has been studied by kinetic measurements, first introduced by Davidson and Fasman,[1] of the formation or disruption of the β structure. From a kinetic study of poly(L-lysine), Wooley and Holzwarth[2] have suggested that two high molecular weight samples (DP = 1000 and 240) form the intramolecular β structure at low concentrations. However, they were not able to confirm the limiting behavior of the rate constant at low concentrations for a low molecular weight sample (DP = 70). The rate constant was concentration dependent for the three samples examined for a wide concentration range. However, this concentration dependence cannot be uniquely interpreted. Snell and Fasman[3] followed a unimolecular conformational change between the α helix and the β structure of the copolypeptides of L-leucine and L-lysine. From the rate constants, they obtained activation energies for the forward and the backward processes and the free energy difference between the α helix and the β structure.

On the other hand, Hartman et al.[4] have reported that light scattering increased with a concentration-dependent rate under the conditions which gave first-order kinetics in the previous studies.[2,3] Hartman et al. have proposed the intermolecular β structure under these conditions and have questioned the previous interpretation.

Kinetic studies have been done on the α–β transition[5] of poly(N^ϵ-methyl-L-lysine) and poly(N^δ-ethyl-L-ornithine) and on the β–coil change of poly(L-tyrosine).[6]

The present problem has been examined by measuring the shape and size of polypeptides in solution. Sarkar and Doty[7] proposed the intramolecular β structure for poly(L-lysine) as early as 1966 based on measurements of sedimentation in a density gradient. Senior et al.[8] have monitored the β–coil transition of poly(L-tyrosine) by molecular weight, radius of gyration, potentiometric titration, and optical activity. Patton and Auer[9] have measured the concentration dependence of the sedimentation coefficient of poly(L-tyrosine) for a dilute concentration range with the aid of ultraviolet absorption of the side-chain chromophore. In this way, they have been the first to show without recourse to kinetic arguments that poly(L-tyrosine) undergoes a transition between the random coil and the unaggregated intramolecular β structure when the concentration is lower than about 2×10^{-4} N[9]. However, this line of approach required measurements on the shape and size at low concentrations, which are not feasible to carry out without the use of the tritiated sample[7] or ultraviolet absorption.[9]

In the present study, the β–coil (disordered state) change of poly[S-(carboxymethyl)-L-cysteine] (poly[Cys(CH_2CO_2H)]) was examined mostly in media containing little or no added salt. We measured the equilibrium or time-independent values of the optical activity obtained 2–3 days after preparation, instead of following the kinetics as used in most of the previous studies. The β–coil change of poly[Cys(CH_2CO_2H)] was affected by pH (or degree of neutralization) and the polymer concentration. Combination of these two factors provided an approach to the present problem different from the direct kinetic approach employed in the previous studies.

Experimental Section

The three samples of poly[Cys(CH_2CO_2H)] used in the present study were obtained by polymerization of the NCA of S-(carbobenzoxymethyl)-L-cysteine followed by debenzylation with HBr. A fractionated sample KM was obtained by ion exchange chromatography (DEAE-cellulose). The amino and carboxyl end groups of this sample are blocked by acetyl and ethylamino groups, respectively. The weight-average molecular weight (degree of polymerization, DP) is $(7.5 \pm 0.3) \times 10^3$ (46 ± 2) as determined from light scattering measurements on Me_2SO solutions. Sample KO43 is an unfractionated sample synthesized as before.[10] Low molecular weight components of sample KO43 were removed by gel filtration (Sephadex G-75). The resulting sample was designated as sample OM. The molecular weight (DP) of sample

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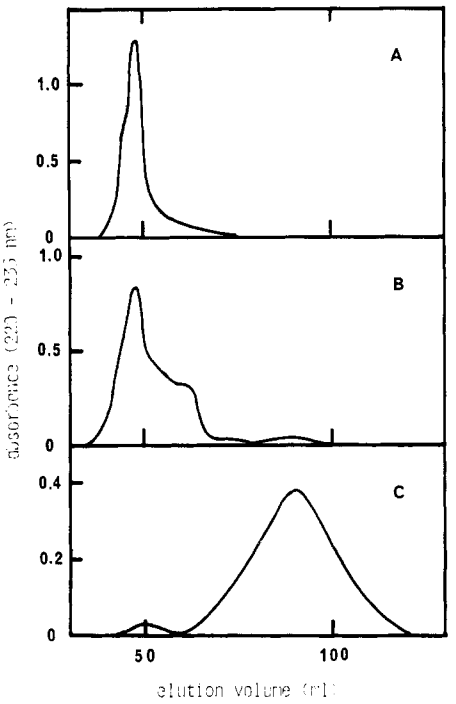


Figure 1. Gel filtration patterns of the samples used: (A) OM; (B) KO43; (C) KM. Gel: Sephadex G-75. Phosphate buffer (pH 7.4, 0.01 M) + 0.2 M NaCl.

OM is 5.27×10^4 (327), as determined in 0.2 M NaCl solutions (pH ~7) by light scattering measurements. The elution patterns of fractionated sample OM, unfractionated sample KO43, and sample KM are given in Figure 1. These results were obtained with a Sephadex G-75 column (85 cm, 1.5-cm i.d.) equilibrated with 0.01 M phosphate buffer (pH 7.4) plus 0.2 M NaCl at ambient temperature (23 ± 2 °C). The flow rate was 20 mL/h. A preparative fractionation of sample KO43 was carried on a Sephadex G-75 column (95 cm, 2.6-cm i.d.) at a flow rate of 60–70 mL/h.

Polymer concentration C_p is expressed in normality (residue molarity). This was determined by titration in the presence of excess salt or on a dry weight basis. To specify a state of a solution, we use the degree of neutralization β in addition to C_p, unless otherwise stated. The degree of neutralization is defined here as the ratio of the amount of added alkali to the total amount of carboxyl groups (both ionized and unionized).

Measurements of pH and CD were made at 25 ± 0.5 °C with a Beckman research pH meter and a Jasco J-40A circular dichrograph, respectively. Measurements of pH and CD were carried out under a nitrogen atmosphere after nitrogen gas was passed through the solutions for 30–90 min. Cells of path lengths 0.20–20 mm were used for CD measurements.

In the present study, solutions were prepared in three different ways. A series of solutions was prepared by the addition of various amounts of NaOH to the stock solutions ($\beta = 0$). The stock solutions were obtained by passing salt-free solutions at a high degree of neutralization ($\beta \sim 0.9$) through a cation exchange column (Amberlite IR-120 or SP-Sephadex). Another series of solutions was prepared by the addition of various amounts of hydrochloric acid to the stock solutions. The degrees of neutralization of these stock solutions were sufficiently high (usually $\beta = 0.7$–0.9) to confirm the disordered state of the polymer in these solutions. The solutions prepared in this second way contain NaCl. Another series of solutions was also obtained by dilution with solvent (water) at a constant β.

For sample OM, CD measurements were also made on solutions of constant pH in the presence of 19 mM $NaClO_4$.

All solutions were kept at room temperature 23 ± 2 °C for about 2 days prior to the measurements of CD and pH.

Results

CD Spectra. In Figure 2 are shown the CD spectra of samples KO43 (solid curve A) and KM (dashed curve B) at $\beta = 0$. These spectra show the predominance of the β structure. The CD spectra characterizing the random coil or the disordered state of poly[Cys(CH_2CO_2H)] are seen in Figure 2 at $\beta = 0.9$. In the case of poly[Cys(CH_2CO_2H)], the residue ellipticity around 200 nm has been shown to be more appropriate than that around 225 nm to monitor the conformational change between the β structure and the random coil.[11] In the present study, the residue ellipticity at 200 nm is used throughout.

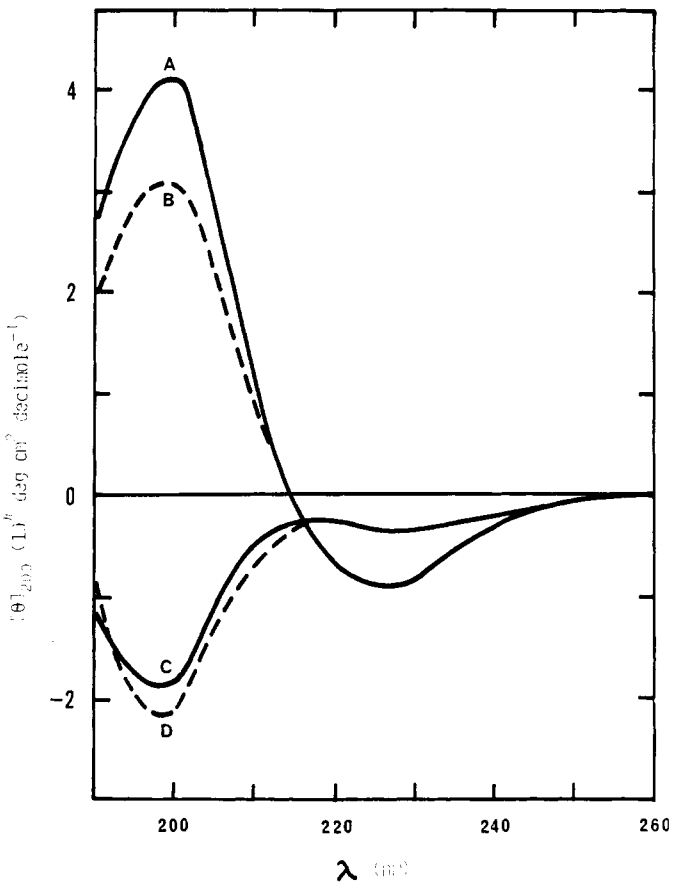


Figure 2. CD spectra of poly[Cys(CH_2CO_2H)]. Polymer concentration is 3×10^{-3} N. Degree of neutralization: (A) 0 (KO43); (B) 0 (KM); (C) 0.9 (KO43); (D) 0.9 (KM).

β Structure by Intermolecular Association. In Figure 3, the residue ellipticities at 200 nm are shown for various degrees of neutralization β at two polymer concentrations in the case of low molecular weight sample KM ($(DP)_w = 46$). Solutions prepared from a stock solution ($\beta = 0$) are represented by circles. Solutions prepared from a stock solution of a high degree of neutralization (mostly $\beta = 0.70$) are represented by triangles. The latter solutions contain sodium chloride; its concentration depends on β and is smaller than the polymer concentration.

At a concentration of 8×10^{-5} N (filled symbols) the β structure is not formed by the addition of HCl even at low degrees of neutralization, as shown by filled triangles. This result indicates that the β structure of sample KM is formed mostly by intermolecular association and that the contribution from the intramolecular process (chain folding) can be safely neglected. When a solution of 8×10^{-5} N is prepared by dilution from a solution (5×10^{-3} N) at $\beta = 0$, however, the β structure is present to a considerable degree. By the addition of various amounts of NaOH to this solution, solutions (8×10^{-5} N) of different degrees of neutralization are prepared. Amounts of the β structure in these solutions are significant and decrease as the degree of neutralization increases. Accordingly, we have a conversion curve defined by filled circles in Figure 3. Thus

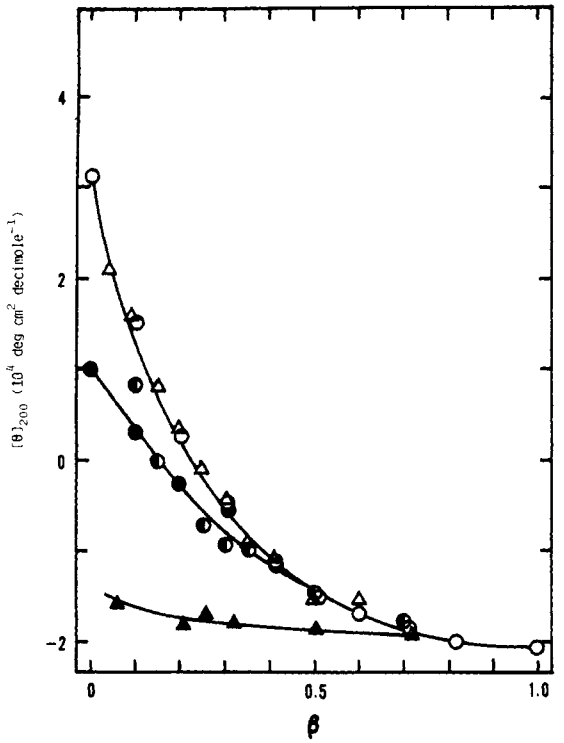


Figure 3. Residue ellipticities of sample KM at 200 nm against the degree of neutralization β. Polymer concentration: 4×10^{-3} N (open symbols) and 8×10^{-5} N (filled symbols). Circles (triangles) refer to the solutions prepared from a solution at $\beta = 0$ ($\beta = 0.7$). Half-filled circles refer to solutions prepared by dilution from the solutions (4×10^{-3} N) of corresponding β.

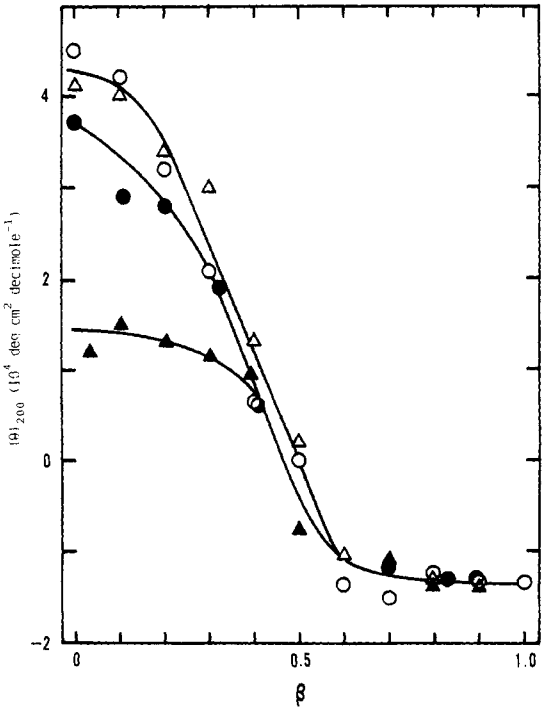


Figure 4. Residue ellipticities of sample OM at 200 nm against the degree of neutralization β. Polymer concentration: 3×10^{-3} N (open symbols) and 8×10^{-5} N (filled symbols). Circles (triangles) refer to the solutions prepared from a solution at $\beta = 0$ ($\beta = 0.9$).

the β–coil change or the association–dissociation process shows a marked hysteresis at this low concentration (filled circles and triangles).

At a concentration of 4×10^{-3} N (open symbols), the β–coil change occurs for a range of β below 0.5–0.6.[12] This assignment is also supported by the titration curve (not shown). As is clearly shown in Figure 3, we are able to show that the dissociation–association process occurs reversibly at this concentration. The reversibility suggests to us that both ways of preparing the solutions (open circles and triangles) are reasonable. The two ways of preparation have been subject to the following suspicion about their validity: the solutions prepared from the solution at $\beta = 0$ may be affected by the extensive aggregation usually found in the stock solution; the solutions prepared from a solution at a high neutralization contain salt and this small amount of salt may affect the properties of the solutions and hence they cannot approximate the solutions with no added salt. It is to be noted, however, that reversibility is established here only with respect to CD and that reversibility about other properties such as aggregation number is not given.

In Figure 3, a significant difference is found between two conversion curves between the β structure and the disordered state: open symbols and filled circles. Undoubtedly, a part of this difference exhibits the effect of concentration on the dissociation–association process. However, the effect of enhanced self-ionization of carboxyl groups due to dilution probably makes a considerable contribution to this difference.

β–Coil Conversion for Higher Molecular Weight Samples. In Figure 4, the residue ellipticity at 200 nm is shown for various values of β at two polymer concentrations in the case of high molecular weight sample OM. The β–coil conversion is reversible at a concentration of 3×10^{-3} N as in the case of the low molecular weight sample. The transition to the β structure begins approximately at $\beta = 0.7$, as supported by the titration curve (not shown). When solutions diluted about 100-fold (3×10^{-5} N) are examined, however, the conversion is found to be partially reversible (filled symbols). This is a marked feature of this high molecular weight sample as compared with the low molecular weight sample.

The effects of polydispersity of a sample should be examined prior to the interpretation of the partially reversible conversion. The β–coil conversion of unfractionated sample KO43 was also examined in detail. The possible difference between these two samples was within experimental error and identical experimental results were obtained for both sample OM and sample KO43. This result provides an example about the effects of polydispersity on the β–coil conversion.

Since the polydispersity of the sample has been shown not to affect the β–coil conversion, at least in the present case, the partially reversible conversion observed at a low concentration in Figure 4 suggests an intramolecular β structure–random coil conversion.

This point can be examined by observing the effects of concentration on the β–coil conversion. To extract the effects of concentration, it is essential to keep the charges of both conformations constant irrespective of the polymer concentration. Generally, values of pH of polyelectrolyte solutions are dependent on the polymer concentration[13–15] if ionic strength is low ($C_p \geq C_s$). Usually, the requirement of a constant charge is satisfied when the degree of neutralization is kept constant. However, this argument cannot be applied to the present case, since it is not certain that self-ionization is completely negligible at the low concentration (10^{-5}–10^{-4} N) encountered here.

On the other hand, charge numbers are uniquely determined by pH if ionic strength is high enough to shield the interactions between polyions. Measurements at a constant pH in the presence of excess salt (19 mM $NaClO_4$) were carried out with sample OM. Solutions of different

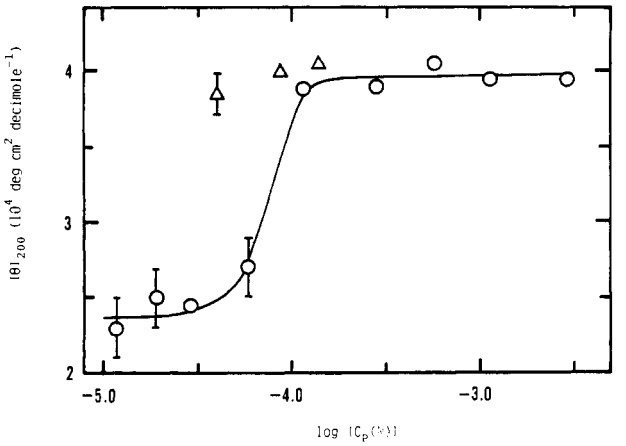


Figure 5. Residue ellipticities of sample OM at 200 nm against the logarithm of the concentration. pH = 4.47 ± 0.05, [$NaClO_4$] = 19 mM. Circles refer to solutions prepared from the solutions of neutral pH by the addition of HCl. Triangles refer to the solutions prepared by dilution from solutions ($C_p \geq 1.2 \times 10^{-2}$ N) of the same pH.

concentrations but a common pH (4.5) were prepared from solutions of corresponding concentrations at neutral pH by the addition of various amounts of HCl. Several solutions were prepared by dilution at a constant pH from a solution. In this case, the pH was readjusted to the prescribed value after dilution. These two series of solutions are represented by circles and triangles in Figure 5.

The residue ellipticities at 200 nm are plotted in Figure 5 against the logarithm of the polymer concentration at pH 4.5. A region of a constant residue ellipticity (about 2.4×10^4 deg cm^2 dmol^{-1}) can be seen for the concentration range below 4×10^{-5} N. First of all, this suggests that aggregation is absent in this concentration range. Accordingly, the β structure in this range is formed intramolecularly, probably by chain folding. For the concentration range above 5×10^{-5} N, the residue ellipticity increases sharply with concentration. This clearly indicates an increase in the β-structure content due to aggregation. The aggregation may occur among the intramolecular β structures and/or intermolecular β structure is formed to a considerable extent. In Figure 5, another region of constant residue ellipticity (about 4×10^4 deg cm^2 dmol^{-1}) is also seen for the concentration range above 1×10^{-4} N. The presence of this region is inconsistent with an interpretation of the data shown in Figure 4 in terms of an intermolecular β structure.

In Figure 5, residue ellipticities of solutions around (4–8) $\times 10^{-5}$ N strongly depend on the method of preparation; a solution prepared by dilution (triangle) contains aggregates while a solution (circle) prepared from a solution of neutral pH by the addition of HCl contains unaggregated intramolecular β structures. Hence, the concentration dependence of the β–coil conversion in 19 mM $NaClO_4$ is irreversible.

The β–coil change represented by filled triangles (3×10^{-5} N) in Figure 4 occurs as a unimolecular transformation between the random coil and the intramolecular β structure without aggregation, as suggested by the results shown in Figure 5. This supposed unimolecular conformational change seems to be far from complete as judged from the level of the residue ellipticity (1.4×10^4 deg cm^2 dmol^{-1}) in Figure 4, which is considerably low even at $\beta = 0$. It is to be noted that this level (1.4×10^4 deg cm^2 dmol^{-1}) is lower than the constant level (2.4×10^4 deg cm^2 dmol^{-1}) observed in the low concentration range at pH 4.5 in Figure 5. Accordingly, self-ionization should be taken into account in this molecularly dispersed state in Figure 4; these solutions ($\beta < 0.3$) in Figure 4 probably have pH higher than 4.5. However, the aggregation in Figure 5 cannot be regarded as caused by a decrease in the extent of self-ionization, because it occurs at a constant pH instead of a constant β.

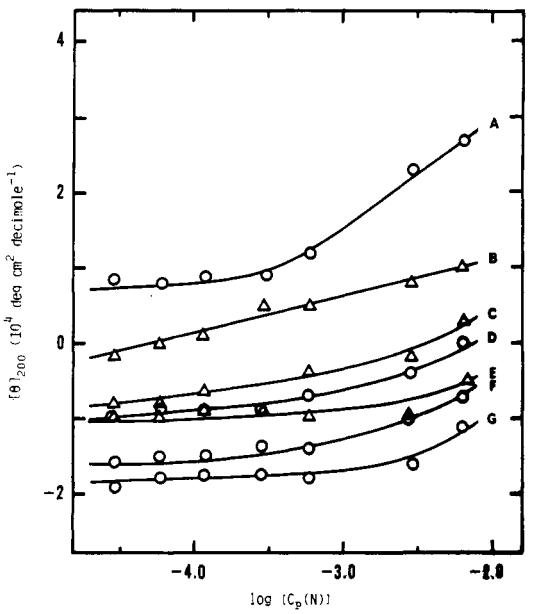


Figure 6. Effect of dilution on the residue ellipticity of sample KM at 200 nm. Degrees of neutralization: (A) 0.10; (B) 0.15; (C) 0.25; (D) 0.30; (E) 0.35; (F) 0.50; (G) 0.70. Circles and triangles represent different ways of preparation of the solutions of the highest concentration (see text).

It is likely, therefore, that the intramolecular β structure has a considerable fraction of disordered conformation at the chain reversal part even at the fully noncharged state and that it is stabilized considerably by aggregation. On the basis of these considerations, the difference between the two transition curves in Figure 4, filled circles and filled triangles, can be best understood as the contribution from aggregation plus different extents of self-ionization, although the two solutions have the same concentration and the same degree of neutralization.

In Figure 4 the difference between the two transition curves, open symbols and filled circles, is now considerably reduced if compared with the corresponding difference for the low molecular weight sample (Figure 3). This is a consequence of the expected effects when molecular weight is increased; the contribution from the intermolecular association is decreased and the β–coil change becomes less dependent on the polymer concentration. At the same time, the self-ionization is greatly suppressed as molecular weight increases.

Effect of Dilution. In the preceding sections the pH-induced β–coil change is examined at two polymer concentrations for three samples. In the present section, the effects of the polymer concentration on the β–coil change are examined by the addition of water to a solution of a given β. In Figure 6, the effects of dilution are shown on a low molecular weight sample at different degrees of neutralization. It is to be noted that the degree of ionization will increase on dilution at a constant β. From Figure 6, it is seen that the β structure is significantly converted into a disordered state on dilution. This behavior indicates an essential role of the association for the β structure of this low molecular weight sample.

It is expected that the method of preparation of the initial solutions from which dilution is started may affect the obtained results. This point is also examined. In

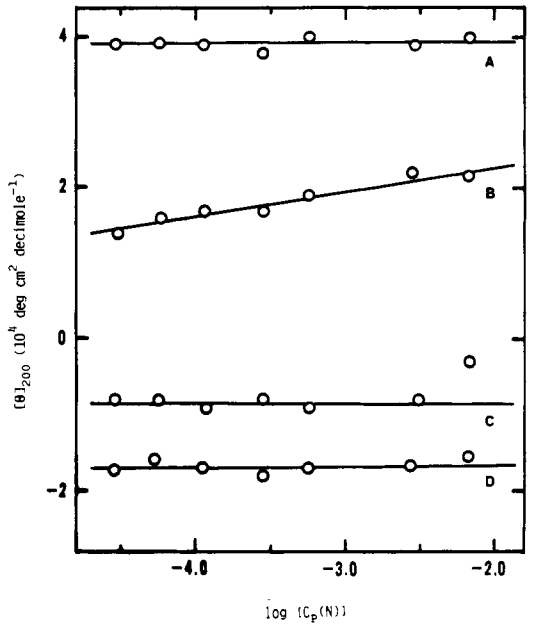
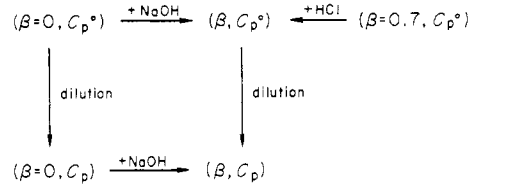


Figure 7. Effect of dilution on the residue ellipticity of sample KO43 at 200 nm. Degrees of neutralization: (A) 0.10; (B) 0.30; (C) 0.50; (D) 0.70.

Figure 6, circles represent a series where the initial solutions are prepared from the state of $\beta = 0$ by the addition of NaOH, whereas triangles refer to another series where the initial solutions are prepared from the disordered state ($\beta = 0.7$) by the addition of HCl. Although a slight difference can be seen between these two series (circles and triangles), no definite conclusion can be drawn on this point at present.

In Figure 6, it is also clear that a complete disruption of aggregates cannot be achieved by dilution within the concentration range examined. The final level of the content of the β structure, attained at the lowest concentration, is dependent on the degree of neutralization.

The interpolated values at a concentration of 8×10^{-5} N are read from Figure 6 and plotted in Figure 3 with half-filled circles. These data fall approximately on the conversion curve defined by filled circles. From this coincidence, it is now established that any given state (β, C_p) on this conversion curve can be reached by at least two different pathways as depicted in the following.



Accordingly, it seems reasonable to regard the state on this conversion curve as an equilibrium or at least as a metastable state. However, as described in the Discussion, this interpretation will meet a difficulty when fluctuations around a given equilibrium state are taken into consideration.

In Figure 7, the effects of dilution are shown on high molecular weight sample KO43. In Figure 4, a reversible change is confirmed for a range of β larger than about 0.3 at 3×10^{-5} N. Correspondingly, a significant conversion into the random coil state on dilution is seen in Figure 7 for $\beta = 0.30$. For $\beta = 0.10$, on the other hand, the β–coil conversion is little affected by dilution. This causes a considerable magnitude of hysteresis at this value of β in Figure 4. This irreversible concentration dependence suggests the presence of aggregation at high concentrations.

Discussion

Irreversible Association–Dissociation. As shown in Figure 3, an irreversible association–dissociation is found at the low concentration of 8×10^{-5} N.[21] It is pertinent to discuss this irreversibility in order to understand the nature of the intermolecular β structure. To facilitate the discussion, we first tentatively make two assumptions and then examine various arguments from the point of view of whether they are against or for one of these assumptions.

Assumption 1. Equilibrium states are represented by filled triangles in Figure 3. In the solutions represented by filled and half-filled circles, the dissociation of aggregates is blocked at some intermediate stage.

Assumption 2. Equilibrium states are represented by filled and half-filled circles in Figure 3. The association of polymers in the solutions represented by filled triangles requires a time longer than the time scale of the present experiment (2–3 days).

Equilibrium states are defined by a set of state variables and are independent of the pathway of how they are prepared. As explained in the section Effect of Dilution, any one of the states on the conversion curve represented by filled and half-filled circles in Figure 3 can be brought through two different pathways. These states thus satisfy a requirement for the equilibrium state, although this result does not provide a sufficient reason for the assignment of the equilibrium. This argument favors assumption 2.

For a simple association–dissociation system, the rate of association depends on the concentration C_p, while the rate of dissociation of aggregates is independent on C_p. As shown by open symbols in Figure 3, the association–dissociation reaches the equilibrium within the time scale of the experiment at 4×10^{-3} N. At the low concentration of 8×10^{-5} N, the rate of dissociation of the aggregates should be similar to that at 4×10^{-3} N. Accordingly, the dissociation of aggregates can occur even at 8×10^{-5} N. This argument also favors assumption 2.

However, an equilibrium state is guaranteed by fluctuations around it. According to assumption 2, the association rate is so small in the solutions represented by filled triangles (β, C_p, $f_1 \sim 1$) that an equilibrium cannot be reached within the time scale of the experiment. Here, f_1 denotes the fraction of unassociated species. For a given solution represented by a filled circle (β, C_p, $f_1 < 1$), however, the association rate should be even smaller, since the concentration ($f_1 C_p$) of unassociated polymer in this solution is lower than that (C_p) in the other solution. Therefore, any fluctuation toward more association seems almost suppressed around the state represented by those filled circles. From this consideration, it can be understood that this state gives time-independent properties. However, it is highly questionable that this state is an equilibrium one. This argument is strongly against assumption 2, while it is consistent with assumption 1. Extensive studies in this area are undoubtedly required.

Chain Folding Mechanism. In the present study, the intramolecular β structure is found to be unstable for the polymer consisting of about 46 amino acid residues. This assignment is consistent with the case of poly(L-lysine) with 70 residues,[2] which has been suggested to be incapable of forming the intramolecular β structure. On the other hand, the intramolecular β structure is proposed for the polymer composed of about 327 residues. However, the intramolecular β structure contains disordered conformations to a considerable degree at a molecularly dispersed state, as suggested from CD measurements. As argued before, the

destabilizing factors responsible for the incomplete intramolecular β structure are considered to be structural rather than to arise from repulsive electrostatic interactions. This point of view is supported by the observation that the intramolecular β structure is greatly stabilized by the aggregation at a constant pH. All these results are rather unexpected if a kind of regular turn structure such as the β or γ turn[16-20] is involved in the folding mechanism. It is likely, therefore, that a kind of chain folding occurs without any turn structure in the present polypeptide and that the chain reversal parts consist of polypeptide chains of little or no regular structure. If this proposal can be extended to the β structure of other synthetic homopolypeptides, then the significance of the presence of the turn structures in proteins will be understood, at least partly, in relation to the chain folding mechanism. It is to be noted that the fraction of the disordered part cannot be decreased by an increase in chain length based on this model. This is a characteristic situation different from the case of the α helix, where the end effects can be nullified as the chain length goes to infinity.

Free Energy of Association. As shown in Figure 3, the conversion between the intermolecular β structure and the disordered state is reversible at 4×10^{-3} N. However, a proper equilibrium constant cannot be defined for the conversion if the number of charges on each species varies with the polymer concentration, which is likely in solutions of weak polyelectrolytes at low ionic strengths. Accordingly, only these data obtained at low charge densities are analyzed based on the reactions 1a,b, assuming that various

$$2A_1 \overset{K_2}{\rightleftharpoons} A_2 \tag{1a}$$

$$A_{i-1} + A_1 \overset{K_i}{\rightleftharpoons} A_i \qquad (i \geq 3) \tag{1b}$$

effects arising from electrostatic interactions can be approximately neglected. Below, we tentatively adopt an indefinite linear association model,[22-24] in which all K_i are equal to a constant K irrespective of i and dimerization constant K_2 is written as σK in terms of a nucleation parameter σ. Further, σ is assumed to be unity, consistent with the highly approximate nature of the present analysis. The free chain concentration C_1 is given by eq 2 in terms

$$KC_t = KC_1 + (KC_1)^2(2 - KC_1)/(1 - KC_1)^2 \tag{2}$$

of K and the total chain concentration C_t.[24] From eq 2, K can be evaluated if C_1 or f_1, defined as C_1/C_t, is known as a function of C_t. Further, we assume that f_1 can be approximated by f_c, the average fraction of residues in the disordered state. From CD data, f_c is given by eq 3. Here

$$f_c = \frac{[\theta]_\beta - [\theta]}{[\theta]_\beta - [\theta]_c} \tag{3}$$

$[\theta]_\beta$ ($[\theta]_c$) denotes the residue ellipticity corresponding to the state where all residues are in the β structure (disordered state). From Figure 4, we take a value of 4.2×10^4 deg cm^2 dmol^{-1} for $[\theta]_\beta$. A value of -1.7×10^4 deg cm^2 dmol^{-1} is chosen for $[\theta]_c$ from Figure 3, after the "charge effect" on CD is taken into account.[11] On the basis of the above approximations and assumptions, we can evaluate the values of K at different degrees of neutralization. Since potentiometric titration data are available for sample KM at 4×10^{-3} N, values of K are plotted against degree of ionization. With a linear extrapolation to zero ionization, we have a value of about 1×10^3 N^{-1} for K. In this limit, the assumption of the reaction among noncharged species will be valid. Since each polymer has 46 residues, $K = 5 \times 10^4$ M^{-1}. The standard free energy change $\Delta G°$ is -6.5×10^3 cal/mol according to eq 4

$$\Delta G° = -RT \ln K \tag{4}$$

For a mole of residue, we have

$$\Delta G°(\text{res}) = -140 \text{ cal/mol.}$$

As is generally noted,[25] this value of the standard free energy change depends on the concentration unit employed. The assumption that $f_1 = f_c$ is equivalent to the assumption that all the residues in the aggregates take the β conformation. This all-or-none model corresponds to one of the simplest pictures, that of the pleated sheet proposed by Pauling and Corey.

Potentiometric titration was carried out at 4×10^{-3} N: the same condition as the data represented by open symbols in Figure 3. A value of $-(3.5 \pm 0.5) \times 10^2$ cal/mol of residue is obtained from the area bounded by two titration curves.[26,27] However, this value cannot be compared with $\Delta G°$(res) evaluated above, since these two free energy changes are not related to the same process.

References and Notes

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