Kinetics of pH-Induced Random Coil–β-Structure Conversion of Poly[S-(carboxymethyl)-L-cysteine]

Kazuhiro Fukada, Hiroshi Maeda,* and Shoichi Ikeda

Department of Chemistry, Faculty of Science, Nagoya University, Nagoya 464-01, Japan.
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ABSTRACT: The rate of the β-structure formation of poly[S-(carboxymethyl)-L-cysteine] becomes faster as the number of charges on the final β-sheets decreases: a decrease of the degree of ionization of the carboxyl groups from 0.25 to 0.20 accelerates the rate by more than 10^4. When the charge densities are low, the β-structure forms through folding of single, isolated chains followed by the aggregation of β-sheets at the final stage of the conversion. The rate of stochastic folding of homopolypeptide chains into the β-structure is shown, for the first time, to be comparable with or faster than that of directed folding of many globular proteins if compared at the same linear charge density. The initiation step is believed not to be rate-limiting since the rates of β-sheet formation are nearly identical irrespective of the β-structure content of the initial state. The whole time course of β-structure formation as well as the reverse conversion to random coils is complex in conformity with the multirelaxation time nature of the reaction.

Introduction

A protein is characterized by its amino acid sequence. On the other hand, every protein possesses a common structural feature, i.e., the polypeptide backbone. Hence, any property of proteins consists of these two contributions. Evaluating one of them leads to the understanding of the other. In studies of homopolypeptides, the effect of the amino acid sequence will be minimum, and hence the general behavior characteristic of the polypeptide backbone is expected to be exhibited in so far as it is successfully extracted from the data on various homopolypeptides.

Folding of single homopolypeptide chains into the β-structure occurs, in principle, at any site on the chain, and hence it is a stochastic process. Therefore, it differs from that of globular proteins, where the folding pathway is determined nearly uniquely by the amino acid sequence.[1-3] It is interesting and also important to know the difference between the folding of homopolypeptide chains and proteins to form the β-structure.

The thermally induced α-helix to β-structure conversion of poly(L-lysine) (PLL) showed single-exponential behavior,[4,5] while its pH-induced coil to β-structure conversion at high temperature showed a lag phase when followed with light scattering or fluorescence.[6] In the case of poly(L-tyrosine) (PLT), a pH-induced coil to β-structure change was successfully described by two exponential terms, and no lag phase was observed.[7] Time domains in these processes were about 10–10^2 min for PLL but 10–10^2 s for PLT. Fast conversions in the 10^{-2}–10^{-1}-s region were observed in a recently reported study on PLT; the results were approximately fitted with two exponential terms, but multirelaxation time character was also observed.[8] Thermal coil to β-structure conversion of poly[S-(carboxymethyl)-L-cysteine] (poly[Cys(CH_2COOH)]) showed multirelaxation time behavior in the time domain 10^2–10^5 s.[9] However, these diverse kinetic behaviors found for different polypeptides are all found for poly[Cys(CH_2COOH)][10] and are explicable in terms of a simple model recently developed.[11]

The time domain obtained in the previous studies suggests that the folding of model polypeptide chains into the β-structure occurs considerably slower than the folding of the proteins. Hence, the role of amino acid sequence in directing the folding pathway may be suggested. It is likely, however, that the rate of folding as well as the stability of the folded chain both depends on the amount of charge carried by the polypeptide chains. Despite this, the effect of pH on the rate of formation of the β-structure has scarcely been examined for PLL. In the case of PLT, the dependence of the rate on pH exhibited a maximum.

In globular proteins, β-sheets are often located in the interior region and thus protected from aggregation, while in model polypeptides, on the other hand, β-sheets are generally exposed to solvent and hence likely to aggregate with each other. It is important, therefore, to follow the reaction by monitoring not only the chain conformation but also the aggregation. A light-scattering study on PLL has shown that aggregation takes place in parallel with the formation of the β-structure.[6]

In the present study, the pH-dependence of the rate of formation of the β-structure as well as concomitant aggregation is examined for poly[Cys(CH_2COOH)] at different polypeptide concentrations. It will be shown that the rate becomes enormously rapid as the charge density of the chain decreases.

Experimental Section

Three samples of poly[Cys(CH_2COOH)] were used in the present study. Their weight-average molecular weights (Mw) and the degree of polymerization (DPw) (in parentheses), determined by light scattering, were 10.1×10^4 (630), 9.0×10^4 (560), and 4.8×10^4 (300). The sample with DPw = 630 is of the same lot as used in the previous study.[12] The other samples were synthesized as reported previously,[13] followed by elimination of a low molecular weight fraction by ultrafiltration using UK-50 or UK-200 membranes (Toyo-roshi Co., Ltd., Tokyo).

The conversion between random coil and the β-structure of poly[Cys(CH_2COOH)] was monitored by means of circular dichroism (CD). Depending on the rates of conversion, kinetic measurements were carried out as follows. When the half-life, $\tau_{1/2}$, of the reaction was longer than 10 s, equal volumes of two solutions of 50 mM $NaClO_4$ + 1–10 mM sodium acetate buffer (one containing the polypeptide at neutral pH and the other at acidic pH without the polypeptide) were mixed with a mixing unit (Model MX-7, Union Giken Company, Osaka) within about 0.1 s and were introduced into the cells for CD measurements by a Jasco J-40A spectropolarimeter. Light paths of the 0.5-, 1.0-, or 2.0-cm cells were used. CD at 205 nm was measured with time constant settings of 1, 4, or 16 s at room temperature (24 ± 2 °C). When values of $\tau_{1/2}$ were smaller than 10 s, on the other hand, kinetic measurements were made with a Jasco J-600 spectropolarimeter having a stopped-flow attachment (SFC-5) with a cell of 1.0-cm optical path. For fast reactions, 16–24 runs were accumulated with time constants of 8 or 16 ms. The reverse conversion from the β-structure to random coils was measured only with the Jasco J-40A. The pH values of the solutions after mixing were measured independently of the kinetic experiments.

The time course of the scattered light intensity after the pH jump was followed with a Chromatix KMX-6 low-angle light-scattering photometer at room temperature. The apparent ag-

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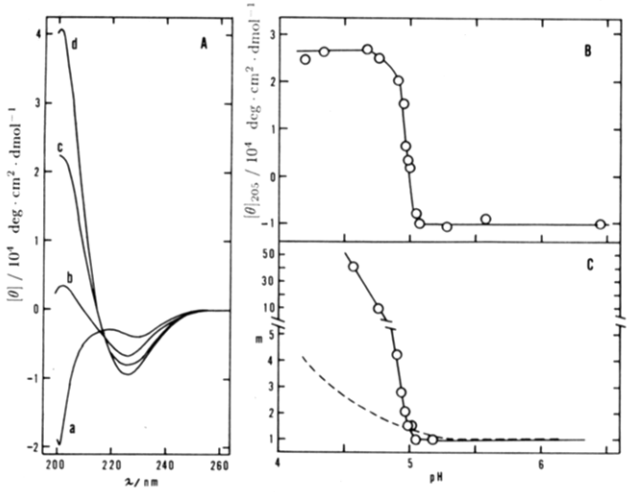

Figure 1. (A) CD spectra of poly[Cys(CH_2COOH)] (DPw = 560) at different values of pH in 50 mM NaCl solutions. Polymer concentration: 1.0×10^{-4} M. Values of pH: (a) 5.58, (b) 5.00, (c) 4.95, and (d) 4.57. (B) pH dependence of residue ellipticities at 205 nm, $[\theta]_{205}$. (C) pH dependence of apparent aggregation number, m. A broken curve in C represents the data of the sample of DPw = 630 taken from ref 12.

gregation number of the polymer was evaluated, as previously described.[12]

Polypeptide concentrations, C_p, changed from 2×10^{-5} to 2×10^{-4} M. Acetate buffer concentrations were correspondingly changed from 1 to 10 mM, in order to keep the ratio to C_p greater than 10. The ionic strengths of the solutions were nearly constant (50–60 mM).

Results

pH-Dependent Coil to β-Structure Conversion and Aggregation at Equilibrium. The CD spectra of a sample (DPw = 560) in 50 mM NaCl solutions at different pH values are shown in Figure 1A. Curves a and d show the CD spectra of the random coil and the β-structure of poly[Cys(CH_2COOH)], respectively. Curves b and c show the intermediate states between the random coil and the β-structure. In this study, residue ellipticity at 205 nm, $[\theta]_{205}$, was chosen as a measure of the extent of conversion between the random coil and the β-structure. Accompanying the coil to β-structure conversion, which occurred at about pH 5.0, $[\theta]_{205}$ varied from $-(8–10) \times 10^3$ to $(2.6–2.9) \times 10^4$, as shown in Figure 1B. Figure 1C indicates the aggregation number of polymers accompanying β-structure formation. Compared to the observations on the sample with DPw = 630 (dashed line),[12] the aggregation number increases sharply in the present study. This is parly due to the difference in chain length and partly due to possible differences in measuring time after the pH values were adjusted. The extent of aggregation depends on time, since, as shown below, the aggregation of β-sheets takes place slowly after they are formed.

pH-Jump Kinetic Measurements. Representative kinetic traces and fitting curves are shown in Figures 2 and 3 at three different values of pH. At pH 4.63, β-formation proceeded in the time range of 10^3 s, as shown in Figure 2. On the other hand, at a slightly lower pH of 4.41–4.48 (Figure 3), coil to β-structure conversion took place in the range of a hundred milliseconds, i.e., extremely fast compared with previous data on β-structure formation of homopolypeptides detected by optical activity.[4–10] Therefore, these results clearly indicate that the rate of β-formation is greatly accelerated as the pH is decreased or as the polypeptide chains carry a small number of charges. Essentially the same kinetic traces as well as pH dependence of rates were obtained with the other two

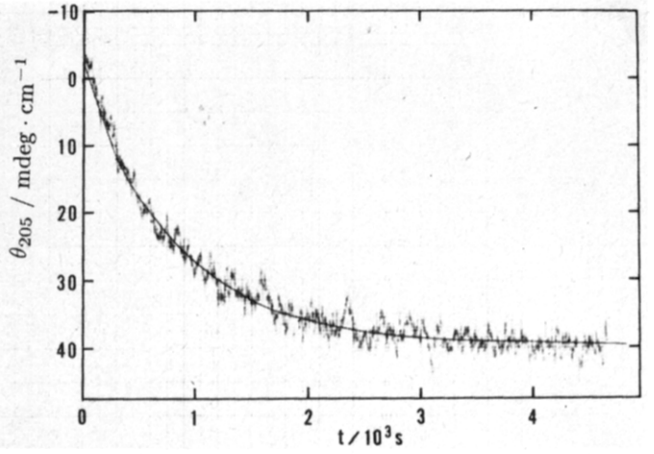

Figure 2. Kinetics of β-structure formation of poly[Cys(CH_2COOH)] in 50 mM $NaClO_4$ + 5 mM solution acetate buffer. DPw = 560. The polymer concentration after mixing is 1×10^{-4} M; pH 4.63. The curve is the nonlinear least-squares fit of the equation $\theta = C_1 - C_2 \exp(-t/\tau)$. The fitting values are $C_1 = 39.5$, $C_2 = 41.7$, and $\tau = 810$.

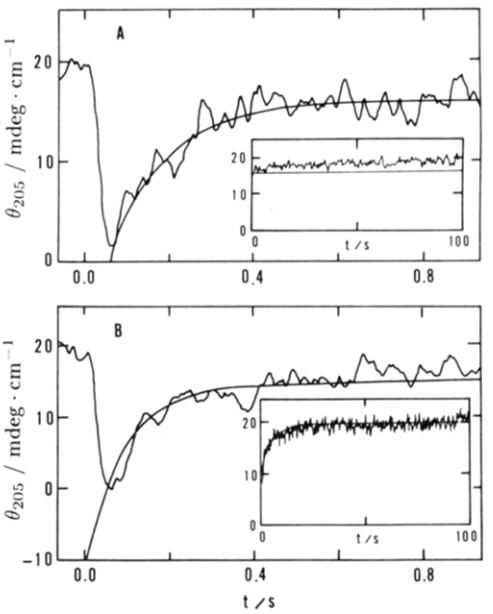

Figure 3. Kinetics of β-structure formation of poly[Cys(CH_2COOH)] in 50 mM $NaClO_4$ + 2 mM sodium acetate buffer. DPw = 560. The polymer concentration after mixing is 1×10^{-4} M. pH 4.41 (A) and 4.48 (B). The curves are the nonlinear least-squares fit of the equations $\theta = C_1 - C_2 \exp(-t/\tau)$ (A) and $\theta = C_1 - C_2 \exp(-t/\tau_f) - C_3 \exp(-t/\tau_s)$ (B). The fitting values are $C_1 = 16.0$, $C_2 = 21.1$, and $\tau = 0.143$ (A) and $C_1 = 20.2$, $C_2 = 26.1$, $C_3 = 6.0$, $\tau_f = 0.095$, and $\tau_s = 8.29$ (B).

samples with DPw = 300 and 630 (not shown). Since the whole time course of the reaction could not be described in terms of the single-exponential term in most cases, we have chosen the half-life, $\tau_{1/2}$, of the coil to β-structure conversion as a measure of the reaction rate.

To correlate the rate of β-formation with charge density, the degree of ionization of carboxyl groups on the polypeptide in the β-structure, α_β, is estimated in terms of the infinite plate model of the β-structure[14] from

$$\mathrm{pH} - \log(\alpha_\beta/1 - \alpha_\beta) - \mathrm{p}K_0 = 0.868 \sinh^{-1}[\sigma(500\pi/\epsilon RTC_s)^{1/2}] \quad (1)$$

where $\sigma = e_0\alpha_\beta/2A_0$, $2A_0 = 33.3 \times 10^{-16}$ cm^2, and $\mathrm{p}K_0 = 3.20$. Also in eq 1, R, T, ϵ, e_0, and C_s represent the gas constant, absolute temperature, dielectric constant of water, elementary charge, and salt concentration (in moles/liter), respectively.

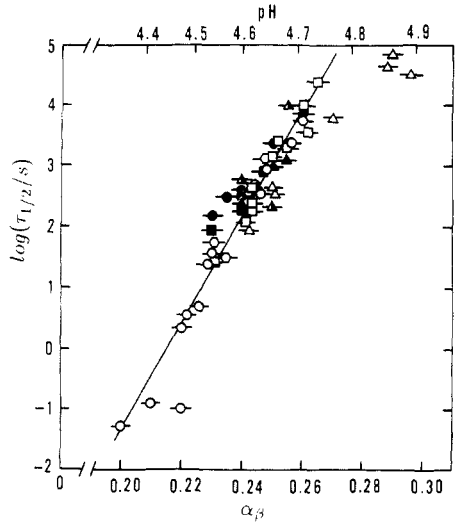

Figure 4. Dependence of the logarithm of the half-life of the reaction ($\tau_{1/2}$) on pH or on the degree of ionization of the polypeptide chains in the β-structure (α_β) in 50 mM $NaClO_4$ + 1–10 mM sodium acetate buffer. Filled, open, and half-filled symbols refer to samples of DPw = 300, 560, and 630, respectively. Different symbols represent polypeptide concentrations after mixing as follows: (▲, △, ▲) 2×10^{-4} M, (●, ○) 1×10^{-4} M, (■, □) 5×10^{-5} M, and (○) 2×10^{-5} M.

In Figure 4, the logarithm of $\tau_{1/2}$ is plotted as functions of pH and α_β for different polypeptide concentrations, C_p. We see that the rate of conversion depends strongly on the charge density of the polypeptide chain. At $\alpha_\beta = 0.20$, the half-life was about 100 ms, comparable with or faster than the characteristic time for the folding of many globular proteins.

Effects of C_p became evident for a range of pH higher than about 4.7; $\tau_{1/2}$ decreased as C_p increased. Even in this pH range, $\tau_{1/2}$ was nearly independent of C_p, if it is lower than 1×10^{-4} M. For pH lower than about 4.7, the rates were independent of C_p in the range $(0.2–2.0) \times 10^{-4}$ M, suggesting the absence of aggregation during β-sheet formation. Since aggregation of polypeptides was observed at this pH, as shown in Figure 1C, it is expected to take place after the β-sheets are formed.

The rates of β-sheet formation of three polypeptides of different chain lengths are also shown in Figure 4; they all coincide. Hence, no chain length dependence was observed in the present study. However, the kinetics of chain folding are expected to differ from those found in this study if the chain length is sufficiently short (shorter than 100).

Aggregation and β-Sheet Formation. In Figure 5 the results of light-scattering measurements at two different values of pH are shown. At pH 4.71, where polypeptide chains carry a considerable number of charges, aggregation took place from the beginning of the β-sheet formation. This strongly suggests that β-sheets are formed principally by aggregation of chains if the pH is high. This is consistent with the observation that $\tau_{1/2}$ depends on the concentration at this pH. However, this does not imply that the β-sheets consist of extended chains. Folding of chains on the surface of aggregates is also possible.[6,10] On the other hand, at pH 4.56 ($\alpha_\beta \simeq 0.23$), more than half of the reaction (ca. 60%) proceeded without significant aggregation, as shown in Figure 5B. At a late stage of the reaction, the aggregation number increased significantly while the amount of β-structure remained almost constant. The aggregation number, m, was about 4 at 3×10^3 s, while the final value of m at this pH was about 50, as shown in Figure 1C. Hence, aggregation takes place significantly

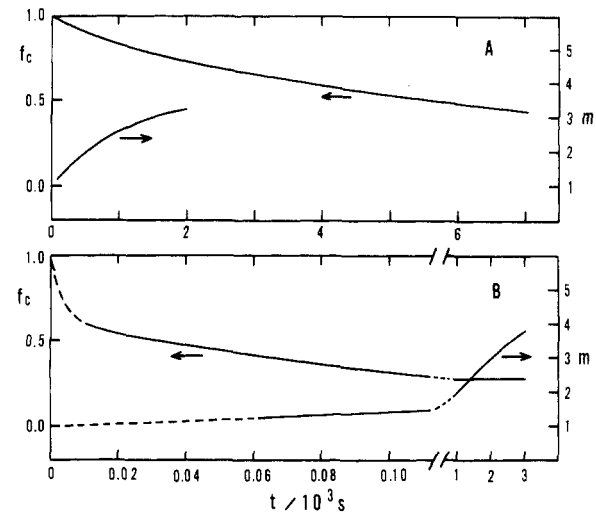

Figure 5. Time course of the fraction of random coil ($f_c = (\theta_{fin} - \theta)/(\theta_{fin} - \theta_{ini})$) and aggregation number (m) at pH 4.71 (A) and 4.56 (B). Polypeptide concentration: 1×10^{-4} M. DPw = 560. 50 mM $NaClO_4$ + 1–5 mM sodium acetate buffer.

in the last stage of β-sheet formation. This reconciles the concentration independence of rates with equilibrium (long time) aggregation numbers (Figure 1C). Aggregation of incompletely folded β-sheets is thus suggested. This mode of association has been found in the previous study.[12]

Analysis of Kinetic Data. In the present study, the total change of $[\theta]_{205}$ associated with any pH jump was well defined from equilibrium measurements, as shown in Figure 1B. When the total amplitude of the signal obtained on kinetic measurements was compared with this equilibrium change, the presence of either an unresolved fast phase or a slow process outside the measuring time domain, or both, was indicated in most cases. The difficultly of covering the whole time domain in a single kinetic measurement is a consequence of the multirelaxation nature of the conformational changes in polymer chains. Only at high pH (>4.7) can the whole process be observed in a single kinetic measurement.

We carried out a nonlinear least-square fitting of the observed time course of the ellipticity, $\theta(t)$, by using two equations. Examples are given in Figures 2 and 3:

formula 1: $$\theta(t) = C_1 - C_2 e^{-t/\tau} \quad (2)$$

formula 2: $$\theta(t) = C_1 - C_2 e^{-t/\tau_1} - C_3 e^{-t/\tau_2} \quad (3)$$

The kinetic amplitudes of a fast unresolved phase, A_1, and of a slow process outside the measuring time domain, A_3, are given as follows:

$$A_1 = \frac{\theta(0) - \theta_{ini}}{\theta_{fin} - \theta_{ini}} \quad (4)$$

$$A_3 = \frac{\theta_{fin} - \theta(\infty)}{\theta_{fin} - \theta_{ini}} \quad (5)$$

Here $\theta(\infty)$ is given as C_1, and $\theta(0)$ equals $C_1 - C_2$ (formula 1) or $C_1 - C_2 - C_3$ (formula 2), and θ_{ini} and θ_{fin} represent the equilibrium values corresponding to before and after a pH jump.

The results are summarized in Table I and Figures 6 and 7. In the pH range higher than about 4.7 (region a), the total change was observed in a time domain covered by single kinetic measurements and could be described by a single-exponential term. In this pH range, the characteristic time constant, τ_2, decreased with increasing concentration, consistent with aggregation as indicated from light-scattering measurements. In region b, corresponding

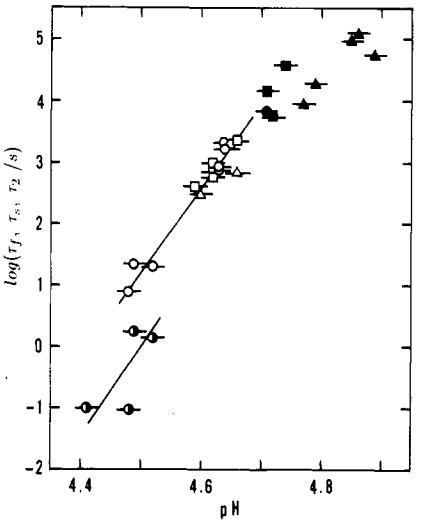

Figure 6. Characteristic time constants obtained from the nonlinear least-squares curve fitting procedure as functions of pH. DPw = 560. Filled, open, and half-filled symbols refer to τ_2, τ_s, and τ_f, respectively. Different symbols represent different polypeptide concentrations as explained in Figure 4.

Table I
Dependence of Kinetic Patterns on pH[a]

pH region		fitting function
(a) 4.7 ≤ pH	$A_1 \simeq A_3 \simeq 0$	$f_c = A_2 e^{-t/\tau_2}$ ($A_2 \simeq 1$)
(b) 4.6 ≤ pH ≤ 4.7	$A_1 > 0$, $A_3 \simeq 0$	$f_c = A_1 + A_s e^{-t/\tau_s}$
(c) 4.45 ≤ pH ≤ 4.6	$A_1 > 0$, $A_3 \geq 0$	$f_c = A_1 + A_f e^{-t/\tau_f} + A_s e^{-t/\tau_s} + A_3$
(d) pH ≤ 4.45	$A_1, A_3 > 0$	$f_c = A_1 + A_f e^{-t/\tau_f} + A_3$

[a] f_c represents the fraction of residues in random coils. A_1 and A_3 represent the contributions from a fast unresolved phase and a slow process following the measured reaction, respectively.

to 4.6 ≤ pH ≤ 4.7, the contribution from the fast process was no longer negligible and was represented by A_1. However, the magnitude of A_1 was so small that the characteristic time associated with the fast process could not be reliably evaluated except at a pH near 4.6. The increase of A_1 around pH 4.6 (Figure 7) is only apparent. This arises from the fact that measurements were carried out with poor time resolution (Jasco J-40A). A considerable part of those A_1 values is expected to be separated as A_f when measured on the other system (J-600). Since the contribution of a slow process (aggregation) could not be resolved from the process described by $A_s e^{-t/\tau_s}$, the effect of the slow process should be considered to be included in τ_s, although the time constants, τ_s, scarcely depended on the concentration. In region c, where the pH is between 4.45 and 4.6, the slow component of the concentration-independent process, most likely corresponding to the folding process, was well resolved as $A_s e^{-t/\tau_s}$ from the slow process represented by A_3. At the same time, the contributions of the fast process were large enough to be clearly resolved as $A_f e^{-t/\tau_f}$. In this way, the chain-folding process was described in terms of at least two time constants, τ_f and τ_s. However, some unresolved fast phase contributing an amount A_1 was still found. In region d, the slow component of the chain-folding process became negligible. The chain-folding process was approximately described by a single-exponential term characterized by τ_f and a fast unresolved phase, A_1. There was considerable contribution from the slow process, A_3. However, a considerable part of A_3 could be resolved as $A_2 e^{-t/\tau_2}$ when measured in the proper time range.

In Figures 6 and 7, the pH dependence of various characteristic time constants is shown. The order of

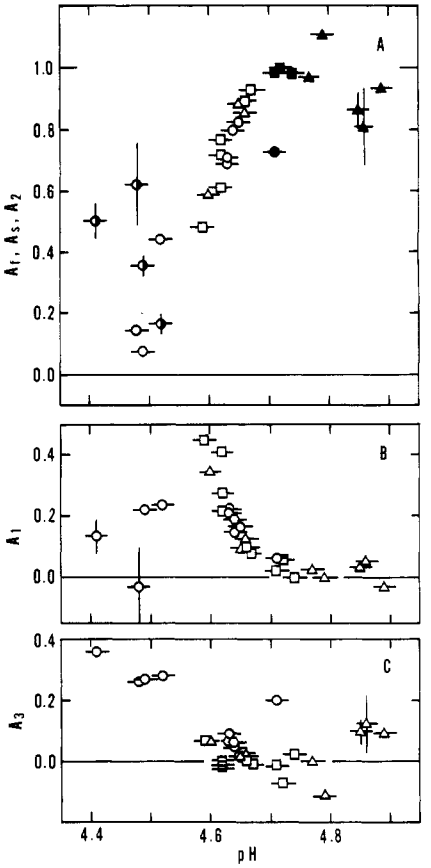

Figure 7. Dependence of kinetic amplitudes of various phases as functions of pH. DPw = 560. Vertical error bars denote standard deviations and are omitted if the latter is comparable with the size of the symbol. (A) A_f, A_s, and A_2 are expressed with half-filled, open, and filled symbols, respectively. (B) A_1 is the fast unresolved phase. (C) A_3 is the slow process outside the measuring time domain. Different symbols represent different polypeptide concentrations as explained in Figures 4 and 6.

magnitude of τ_f is 0.1–1 s, which is very small compared with previously reported values for β-sheet formation. In relation to the unresolved fast phase, it is to be noted that some changes of absorption in the ultraviolet region in the time range 10–100 ms have been reported on PLT.[8] Since the dependence of the rate on pH differs very much between PLT and poly[Cys(CH_2COOH)], it is uncertain whether these two fast processes of the respective peptides can be related to each other.

The fitting procedure in terms of eq 2 gave unique results in most cases. These were similar to those obtained from the simple fitting version,[15] i.e., a fitting procedure to the kinetic data alone without recourse to equilibrium data. The fitting procedure in terms of eq 3 was carried out, on the other hand, under the limitation that characteristic times were not allowed to deviate from the results from the simple version by more than 20%. Under this limitation, the best fit was successfully obtained. Consequently, the results obtained in the present study were similar to those previously reported[15] with respect to the characteristic times. On the other hand, the magnitudes of A_f, A_s, and A_2 differed considerably between these two fitting procedures. In the studies on PLL and PLT, critical examination of the agreement of kinetic amplitudes with equilibrium data was not presented. Although the characteristic times or rate constants reported in these studies are essentially correct, it is uncertain in these studies whether other contributions such as unresolved fast phase or slow processes outside the measured time domain, exist or not.

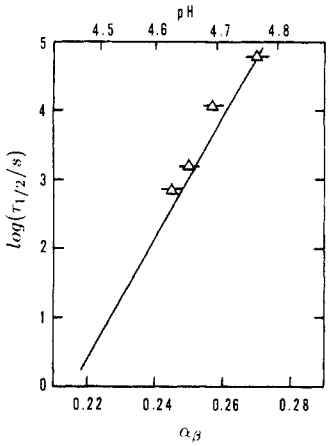

Figure 8. Logarithm of half-life, $\tau_{1/2}$, of the β-structure formation from an initial state belonging to the conversion region as functions of pH or the degree of ionization of the final β-structure (α_β). Initial state: pH 4.99, $[\theta]_{205} = 2.0 \times 10^3$ deg cm^2 dmol^{-1}, $f_\beta = 0.30$. Polypeptide concentration: 2×10^{-4} M. DPw = 560. A straight line represents the results shown in Figure 4.

pH-Jump Kinetics from the Coil to β-Structure Conversion Region. In the preceding sections, the kinetics of β-sheet formation from the random coil are described. The conversion generally consists of an initiation step (or nucleation step, if appropriate) in addition to a propagation step. It is pertinent to examine whether the initiation step is rate-limiting in comparison with the propagation step. We therefore carried out kinetic measurements of β-sheet growth starting from chains consisting of several residues in the β-structure conformation. The results are indicated in Figure 8, where values of $\tau_{1/2}$ are given as functions of the final pH or degree of ionization. The initial pH before the pH jump was 4.99, corresponding to a fraction of the β-sheets of about 0.3, according to Figure 1B. Hence, it was expected that a sufficient amount of nucleus was present in the initial state. The values of $\tau_{1/2}$ were defined as the half-life of the conversion from the initial state ($f_\beta \sim 0.3$) to the final state ($f_\beta = 1$); i.e., at $t = \tau_{1/2}$, $f_\beta \sim 0.65$. The rates were nearly identical with those observed in the conversion from completely random coils. The result shows that the nucleation step plays little or no role in the folding process of poly[Cys(CH_2COOH)]. The result might be explicable with a two-state (all-or-none) model. According to this model, however, the kinetics of the conversion should be described with a single-exponential term, which is contrary to the finding in this study. In this context, it is to be noted that a cooperative unit greater than six residues has been found for the coil–β conversion.[9]

Reverse Reaction (β-Sheet to Random Coil Conversion). Reverse reactions from the β-sheet to random coils were also examined with two samples of different chain lengths. The results, shown in Figure 9, indicate that the conversion took place in at least two steps. An initial rapid reaction was not detected and hence is not shown in Figure 9. Only the second step is shown there. As the final pH increased, the contribution from the initial fast reaction step increased. Consequently, the rate of initial step strongly depended on the number of charges, just as found for the rate of β-sheet formation in the preceeding sections. Similar results were obtained irrespective of the chain lengths of the samples.

As shown in Figure 9, most data for unfolding reactions cannot be described in terms of one or two expontentials. It is to be noted that in the kinetic studies on coil to β conversion described in the proceeding sections, polypeptide chains were converted to the complete β-structure

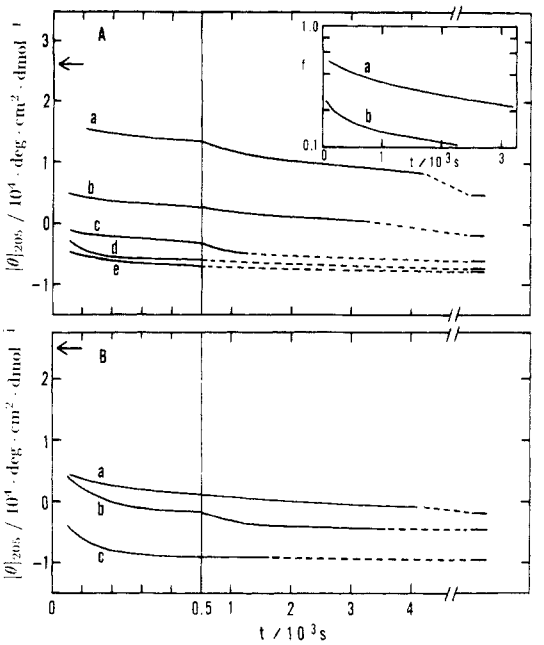

Figure 9. Kinetics of β-structure to random coil conversion in 50 mM $NaClO_4$ + 2 mM sodium acetate buffer. Arrows indicte initial values of $[\theta]_{205}$. (A) DPw = 560. Polypeptide concentration: 1.5×10^{-4} M. Initial pH 4.65. Final pH (a) 4.97, (b) 4.98, (c) 5.04, (d) 5.05, and (e) 5.07. Inset: A semilogarithmic plot of the unreacted β-structure fraction ($f = (\theta_{fin} - \theta)/(\theta_{fin} - \theta_{ini})$) versus time for the data at (a) pH 4.97 and (b) pH 4.98. (B) DPw = 300. Polypeptide concentration: 2.0×10^{-4} M. Initial pH 4.64. Final pH (a) 5.01, (b) 5.06, and (c) 5.13.

with different amounts of charges depending on the final pH, i.e., $f_\beta = 1$ at the final state. On the contrary, in the study on β to coil conversion described in this section, various extents of the conversion were allowed in the final state. For the case of complete conversion, i.e., at pH higher than 5.1, the contribution from the slow step became small and the whole reaction proceeded rapidly. In the other case, reverse reaction (the β-structure formation) could not be ignored. Hence, slow kinetics were predominant, which indicated the random walk nature of the unfolding process.

Discussion

Pathways of the β-Sheet Formation. Kinetic pathways from fully ionized random coils to β-sheets suggested from the present study are schematically depicted in Figure 10. At low charge densities and low concentrations, folding of isolated chains occurs (step A). The β-sheets thus formed gradually associate with each other at the late stage of the conversion (step B). This situation was first observed in the present study. This observation, association of folded chain β-sheets, is consistent with the mode of association of the β-sheet found in a previous study.[12] On the other hand, if concentrations are higher than ca. 2×10^{-4} M, the β-sheet formation occurs through both chain folding and aggregation of extended chains (step C). One interesting observation was that at high charge densities β-sheet formation through aggregation was favored (step C). This is because folding of single chains of high charge densities occurs with difficulty. For a portion of β-strands to be formed, the linear charge density (α_β) of the portion should be lower than that of the remaining part (α_c). This adjustment of charges is possible because hydrogen ions can move about the sites, a characteristic property of weak electrolytes. When the charge density is high, there is almost no room for this chrage rearrangement to occur for single chains. On association of two chains, the rearrangement becomes easier than for

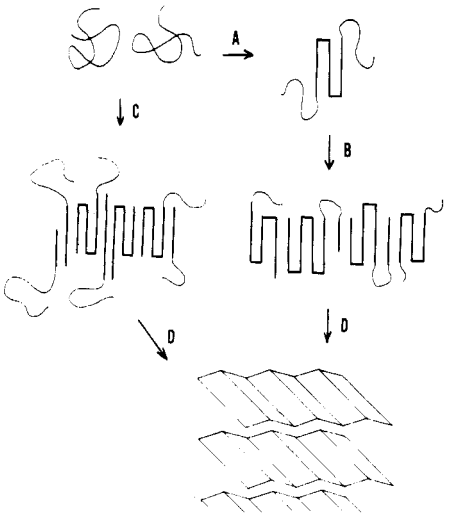

Figure 10. Schematic representation of the pathways of β-structure formation. (A) Chain folding of randomly coiled polyions into molecularly dispersed β-sheets (not complete). (B) Aggregation of β-sheets probably in the side-by-side mode. (C) Formation of the β-sheets through aggregation among extended chains and folded chains. (D) Face-to-face aggregation (stacking) leading to three-dimensional aggregates.

isolated chains. As the final stage of the β-structure formation, face-to-face aggregation or stacking of β-sheets occurs (step D).

Under the condition that aggregation is detected at equilibrium, chain folding of single isolated chains takes place at an early stage of the conversion, and the rate is independent of concentration. In other words, kinetic criteria for the absence of aggregation based on concentration-independent rates differ considerably from those for equilibrium. The absence of aggregation at equilibrium is not necessarily guaranteed.

Dependence of the Rate on the Number of Charges on the Polypeptide Chain. In the present study, the rate of β-sheet formation increases greatly with a small decrease in the number of charges. This is in contrast with the observation of PLT, where the rate goes through a maximum as the number of charges decreases. This dependence of the rate on charge indicates that the rate of β-sheet formation increases with its stability.

Finkelstein has proposed a model correlating the rate of β-sheet formation with its stability.[16,17] Only a few experimental data can be compared with this model at present. The temperature-induced α-helix to β-sheet conversion of PLL has been referred to in this respect. Mere comparison of those data with theoretical prediction seems inadequate, however, since at elevated temperatures not only the stability of the β-structure of PLL but also the rate constants of the various elementary steps are expected to increase.

The present results are consistent with Finkelstein's prediction, since the stability varies at a constant temperature. However, the present results do not necessarily provide experimental support for the model. According to the model, the rate-limiting step is in the formation of a stable nucleus. The stability of the nucleus, a two-stranded β-hairpin, generally parallels that of the β-structure. This is the basis of the correlation in the theory between the rate and the stability.

In the present study, the rates of β-sheet growth observed with pH jumps from the initial states populated by significant amounts of β-structure were essentially identical with those from the random coil state. Consequently, nucleation does not play a crucial role in the kinetics of β-sheet formation. This is inconsistent with the fundamental assumption of Finkelstein's model. The present results suggest that the rate of propagation or strand growth itself is accelerated as the number of charges decreases.

As shown in Figure 4, the half-life of β-sheet formation is of the order of 0.1 s at $\alpha_\beta = 0.20$, which corresponds to about −112 charges for a polypeptide chain with DPw = 560. The amount of net charge of most globular proteins is considerably smaller than 100. In particular, β-sheets are often located on the inside of proteins where net charges are negligible. Consequently, if compared at the same linear charge density, the rate of stochastic folding of homopolypeptide chains to form β-sheets can be much faster than that of directed folding of globular proteins having β-sheets.

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Registry No. Poly[*S*-(carboxymethyl)-L-cysteine] (homopolymer), 29433-95-2; poly[*S*-(carboxymethyl)-L-cysteine] (SRU), 31851-29-3.

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