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Diverse Kinetic Behaviors of the β-Structure Formation. A Study on Poly(*S*-carboxymethyl-L-cysteine)

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Synopsis. The pH jump kinetics of random coil-to-β-structure convertsion of poly(*S*-carboxymethyl-L-cysteine) were examined on two samples of degree of polymerization (DP) of 330 and 560. Various kinetic behaviors were observed depending on polymer chain length and concentration: single exponential with or without a lag and two exponential behavior. In unbuffered solutions, the rate enhancement of β-formation was confirmed.

Kinetics of the β-structure formation of polypeptides have been studied mainly on two polypeptides; poly(L-lysine)(PLL)[1-5] and poly(L-tyrosine) (PLT).[6,7] Conversion from the α-helix to the β-structure of PLL with degree of polymerization (DP) 240 or 1000 by a temperature change followed first-order kinetics and the rate was independent of concentration below about 10—20 mg L^{-1}.[2] In the kinetics of coil-to-β conversion of PLL (DP=700) induced by an isothermal pH jump at high temperature, however, there was a slow initiation step and the progress of reaction was sigmoidal.

In the case of β-formation by pH jump of PLT, the kinetics were described in terms of superposition of two exponential terms without any sigmoidal behavior.

These results show that two polypeptides behave differently with respect to kinetics of chain folding. This aspect was already pointed out[6] and hence diverse kinetic behaviors are ascribed to different kinds of side chains of the polypeptides. However, it will be shown in the present study that various kinetic behaviors previously found on PLL and PLT were all observed on the kinetics of pH induced coil-to-β conversion of poly(*S*-carboxymethyl-L-cysteine) (poly[Cys(CH$_2$COOH)]), another well characterized model β-polypeptide.[8-10]

Experimental

Average degrees of polymerization (DP) of the two samples of poly[Cys(CH$_2$COOH)] used in the present study were 330 and 560. Residue ellipticity at 210 nm, $[\theta]_{210}$, varied from $-(4—5)\times10^3$ to $(1.0—1.1)\times10^4$ and that at 205 nm, $[\theta]_{205}$, varied from $-(8—10)\times10^3$ to $(2.6—2.9)\times10^4$ accompanying to coil-to-β conversion. The conversion occurred at about pH 4.9 in 50 mM sodium salt aqueous solutions (1M=1 mol dm^{-3}). We used $[\theta]_{210}$ or $[\theta]_{205}$ as a measure of the β-formation. Circular dichroism (CD) was recorded on a Jasco J-40A circular dichrograph at 25(±0.1) °C, using cells of 2, 10 and 20 mm light paths.

Kinetics of the β-formation were carried out as follows. The solution in a test tube containing poly[Cys(CH$_2$COOH)] and 50 mM NaClO$_4$, with or without sodium acetate buffer (pH≈7), was brought to acid pH (between 4.7—4.9) by the addition of a small amount of 0.1 M HCl followed by immediate mixing. It took only a few seconds to attain complete mixing. A part of the mixed solution was introduced into the cell and $[\theta]_{210}$ or $[\theta]_{205}$ was continuously recorded for about 1.5×10^3 s. The earliest reliable readings were obtained at about 15 s after the pH jump. Afterward, CD spectra (200—260 nm) were measured at selected time intervals until the reaction was completed. The rest of the solution was used for pH measurement. An Iwaki M 225 pH meter was used to determine the pH when the reaction were completed.

Results and Discussion

Diverse Kinetic Behaviors of the β-Formation of Poly[Cys(CH$_2$COOH)]. Kinetic study of the β-formation in acetate buffer solutions was performed at two polymer concentrations (C_p) on the sample of DP=330 (see Fig. 1). The dependence of the rate of β-formation on pH is shown in Fig. 1(a). At a C_p of 5×10^{-4} M (80 mg L^{-1}), the β-formation proceeded faster as the pH became lower, where β-structure carried a smaller number of charges. The effect of concentration can be seen in Fig. 1(b), where β-formation is slower at the polymer concentration of 1×10^{-4} M (16 mg L^{-1}) than at 5×10^{-4} M. Figure 1(b) further indicates that the β-formation at $C_p=1\times10^{-4}$ M hardly proceeds within 5×10^3 s (a time lag) and the whole kinetic curve is sigmoidal.

These kinetic data were analyzed as follows. The extent of unreacted fraction after pH jump is given with Eq. 1 as a function of time.

$$f(t) = ([\theta]_{210}(\infty) - [\theta]_{210}(t))/([\theta]_{210}(\infty) - [\theta]_{210}(0)) \quad (1)$$

Here, $[\theta]_{210}(t)$ denotes the residue ellipticity at time t, and $[\theta]_{210}(\infty)$ is the value at equilibrium. Semilogarithmic plots of f versus time are indicated in the insets of Fig. 1(a) and (b). As shown in the inset of Fig.1 (a), at $C_p=5\times10^{-4}$ M the time course $f(t)$ could not be fitted with a single exponential term but by the superposition of two exponential terms shown below

$$f(t) = A\exp(-t/\tau_1) + (1 - A)\exp(-t/\tau_2) \quad (2)$$

Here, τ_1 and τ_2 are the phenomenological time constants for the fast and slow processes, and A is the amplitude of the fast process. It was confirmed that the two-term exponential decay curves gave better fit to all the experimental data shown in Fig. 1(a) than single exponential curves. This kinetic pattern is in accord with the kinetics of PLT.[6,7]

At $C_p=1\times10^{-4}$ M, on the other hand, the reaction approximately follows a single exponential decay after a time lag (see inset of Fig. 1(b)). Single exponential decay with a lag was also found in the isothermal pH jump kinetics of PLL. It is to be noted that the lag in the case of PLL was obtained with light scattering and fluorescence but not with optical activity as was done in the present study.[5] It was expected that at very dilute solutions such as

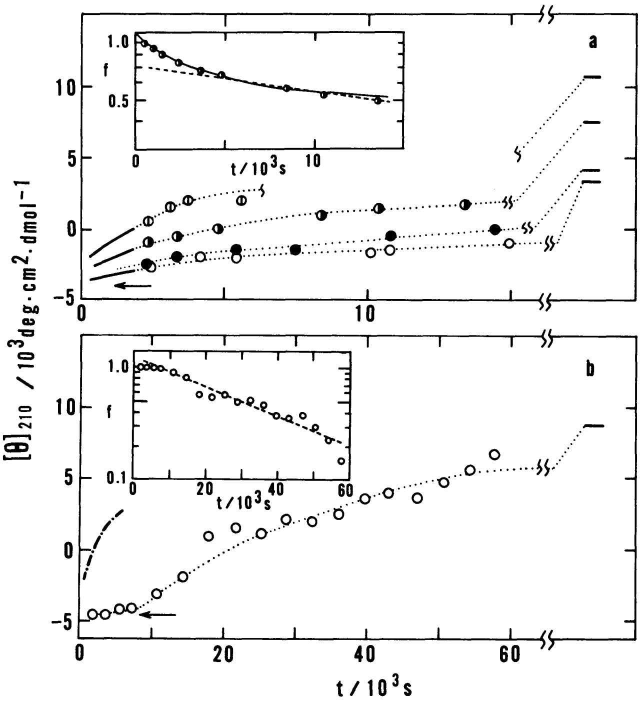

Fig. 1. Kinetics of the β-formation of the sample of DP=330 in 20 mM sodium acetate buffer +50 mM $NaClO_4$. Values of $[\theta]_{210}$ are represented with various circles and with solid thick lines for the initial stage where measurements were carried out continuously. Arrows indicate the initial value of $[\theta]_{210}$.

(a) $C_p=5\times10^{-4}$ M; pH=4.90 (○), 4.87 (●), 4.84 (◑), and 4.74 (⦶). Inset: A semilogarithmic plot of the unreacted fraction f (defined by Eq. 1) versus time of the data at pH 4.84. A solid line indicates the two-term exponential curve, i.e., $f=0.42\exp(-t/2700)+0.58\exp(-t/140000)$. A broken line indicates the single exponential decay curve fitted to the data of long time region.

(b) $C_p=1\times10^{-4}$ M; pH=4.76. A chain line indicates the data at $C_p=5\times10^{-4}$ M (pH=4.74). Inset: A semilogarithmic plot of f versus time. A broken line indicates the single exponential decay curve with a lag time of 5×10^3 s.

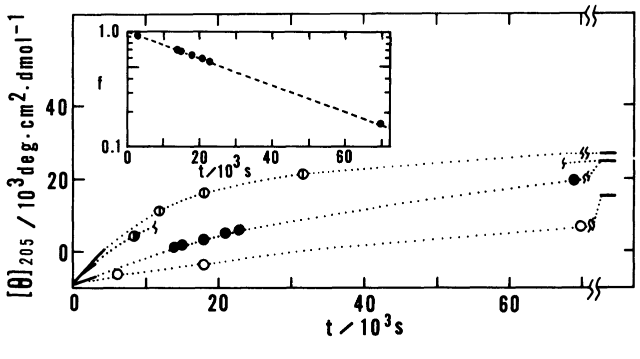

Fig. 2. Kinetics of the β-formation of the sample of DP=560 at different values of pH and concentrations. $C_p=2\times10^{-4}$ M; 10 mM sodium acetate buffer +50 mM $NaClO_4$: pH=4.86 (○) and 4.79 (◑). $C_p=5\times10^{-5}$ M; 2 mM sodium acetate buffer +50 mM $NaClO_4$: pH=4.74 (●) and 4.71 (⦶). Inset: A semilogarithmic plot of f versus time for the data at pH 4.74 ($C_p=5\times10^{-5}$ M).

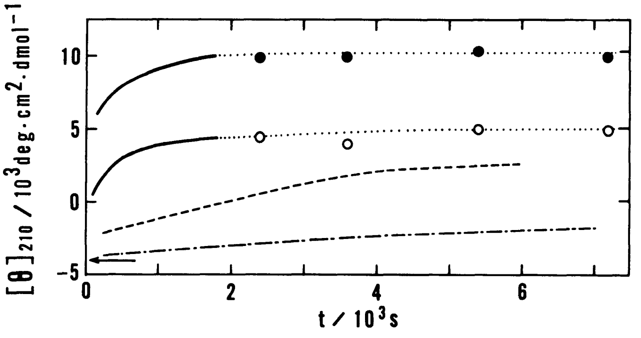

Fig. 3. Kinetics of the β-formation in unbuffered solutions at $C_p=5\times10^{-4}$ M in 50 mM $NaClO_4$ for the sample of DP=330. (○) pH 4.90 and (●) pH 4.80. Solid thick lines represent the values of $[\theta]_{210}$ for initial stage where measurements were carried out continuously. The broken (chain) curve represents the kinetic data at similar concentration in acetate buffer of similar ionic strength at pH 4.74 (4.90). Those data are taken from Fig. 1(a) and given for the sake of comparison.

1×10^{-4} M, β-formation of poly[Cys(CH_2COOH)] proceeded by two-step mechanism of slow nucleation followed by fast growth.

In Fig. 2, kinetic data of the sample of DP=560 are summarized for different pH and concentrations. No lag was observed even at lower concentration of 5×10^{-5} M (8 mg L^{-1}) for this long chain sample. All kinetics shown in Fig. 2 followed the first-order rate law, as indicated in the inset for an example. The first-order kinetic behavior is consistent with the case of thermally induced $\alpha\rightarrow\beta$ conversion of PLL.[2-4] Thermal coil-to-β conversion of poly[Cys(CH_2COOH)] showed kinetic behavior too complex to be described with two exponential terms.[12]

From these results, it was confirmed that various kinetic behaviors previously found on PLL and PLT were all observed in the β-formation of poly[Cys(CH_2COOH)] depending on polymer chain length and concentration: single exponential behavior with or without a lag and two exponential behavior. Therefore, we expect that diverse kinetic behaviors accompanying the β-formation originate rather from diverse aspects of the kinetics of polypeptide chains in general than from different kinds of side chains as has been supposed to be. It is to be noted that the β-formation of homopolypeptide chains consists of many elementary steps and hence various kinetic behaviors manifest themselves depending on which step is rate-limiting. In addition, the polydispersity of samples may possibly contribute to the diverse behaviors. A simple kinetic theory explicable of diverse kinetic patterns of intramolecular β-formation has been recently presented.[13]

Rate Enhancement of the β-Formation in Unbuffered Solutions. In Fig. 3, the rates of β-formation in unbuffered solutions are compared with those in buffer at similar conditions. At the same pH of 4.90, the rate of the β-formation is faster in the absence of acetate buffer. Within 4×10^3 s the β-formation is completed at both pH 4.80 and pH 4.90 in the case of unbuffered solution.

It was confirmed that acetate buffer had little effect on the stability of the β-structure of poly[Cys-(CH_2COOH)],[11] hence its specific effect on the kinetics can be ignored.

The observed rate enhancement could be explained in terms of either or both of the two mechanisms: the seeding effect and the different pathways. The formation of seed may occur as follows. When 0.1 M HCl is added to and mixed with the polymer solution, the added 0.1 M HCl will be dispersed in a number of small drops, hence microscopic inhomogeniety with respect to pH will result. In the presence of buffer, this inhomogeniety disappears rapidly due to the fast diffusion of both H^+ and acetate ions. In the absence of buffer, however, this equilibration process takes a long time. At the early stage of the process, the conversion of polymer molecules from random coils to the β-structure nuclei takes place in the interface region between the low pH small drops and the polymer solution. There remain these nuclei, more or less, after pH becomes uniform. They play the role of the seed in the folding of unfolded molecules.

On the other hand, it is likely that the reaction proceeds via different pathways for buffered and unbuffered solutions. While the coil-to-β conversion proceeds at constant pH in buffered solutions, it proceeds at nearly constant charges in the case of unbuffered solutions (random coil polymers are protonated at first by the added acid, and then converted to β-structure under the constant charges).

We carried out pH jump kinetic experiments with unbuffered solutions using a mixing unit for stopped-flow apparatus (MODEL MX-7, Union Giken Company, Osaka) which mixed solutions at most within 0.1 s. The rate enhancement was again observed by means of this device nearly to the same extent as with manual mixing. This result supports the latter mechanism rather than the former, unless the β-structure nuclei were formed within 0.1 s.

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