Molecular Weight and Conformation of Poly(*S*-[(3-hydroxypropyl)-carbamoylmethyl]-L-cysteine) in Various Solvents

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ABSTRACT: The number-average and weight-average molecular weights of seven samples of poly(*S*-[(3-hydroxypropyl)-carbamoylmethyl]-L-cysteine) (poly(Cys[CamPrOH])) have been determined by measurements of osmotic pressure of 0.1% CH₃COOH solutions and light scattering of hexafluoro-2-propanol solutions, respectively. A strong tendency of poly(Cys[CamPrOH]) to form the β -conformation is observed in various solvents, including CH₃COOH–H₂O, DCA, H₂O, and D₂O. When dissolved in H₂O at 70°C, poly(Cys[CamPrOH]) having molecular weight lower than 18,000 is randomly coiled and that having molecular weight higher than 20,000 is subject to the thermally-induced β -coil transition. When dissolved in D₂O at 70°C, poly(Cys[CamPrOH]) undergoes the thermally-induced β -coil transition, irrespective of the molecular weight. D₂O stabilizes the β -conformation more effectively than does H₂O, by the change in the structure of water rather than by the substitution of D for H on the polypeptide.

KEY WORDS Poly(*S*-[(3-hydroxypropyl)-carbamoylmethyl]-L-cysteine / β -Conformation / β -Coil Transition / Molecular Weight / Hexafluoro-2-propanol / Deuterium Oxide / Osmotic Pressure / Light Scattering / Circular Dichroism /

In our previous studies^{1,2} we have demonstrated that poly(*S*-[(3-hydroxypropyl)-carbamoylmethyl]-L-cysteine) (poly(Cys[CamPrOH])) can be prepared from poly(*S*-carbobenzoxymethyl-L-cysteine) and that it takes on the β -conformation both in solid film and in mixtures of H₂O with D₂O or aliphatic alcohols of low H₂O composition. Specifically, poly(Cys[CamPrOH]) dissolved in D₂O has shown temperature dependence of circular dichroism, indicating the formation of β -conformation at low temperature. On the other hand, the circular dichroism of poly(Cys[CamPrOH]) in H₂O remains unaltered by temperature and its conformation is randomly coiled. The present study reveals, however, that the molecular weight of the sample used was lower than the threshold values in order for the conformational change to occur in H₂O.

In the present work, we investigate the effect of molecular weight on the conformation of poly(Cys[CamPrOH]) in H₂O or D₂O, by measuring circular dichroism. To determine molecular weight, we measure osmotic pressure of aqueous

CH₃COOH solutions and also light scattering of hexafluoro-2-propanol (HFIP) solutions. The molecular conformation and the association of poly(Cys[CamPrOH]) in various solvents including dichloroacetic acid (DCA) are examined. It is found that a temperature-dependent β -coil transition of poly(Cys[CamPrOH]) is induced even in H₂O if the molecular weight is higher than about 20,000, while the same transition occurs in D₂O for all samples examined.

EXPERIMENTAL

Materials

Six samples of poly(Cys[CamPrOH]) were prepared by a slight modification of the method previously described.² The amidation reaction was performed overnight in the presence of chloroform at 60°C, and the polymer was precipitated by the addition of excess chloroform. A sample TX-4071 had been prepared previously. Some of the samples, which proved to have high molecular weights, were

insoluble in H_2O as well as in D_2O , at the concentrations sufficiently high for circular dichroism measurements, when the dissolution was made at room temperature.

DCA was redistilled *in vacuo* and used immediately. CH_3COOH of reagent grade was purchased from Hayashi Jun'yaku Kogyo Co. D_2O (99.75% pure) and HFIP of spectrograde reagent were obtained from Merck. H_2O was redistilled in a glass still from alkaline KMnO_4 .

Methods

The osmotic pressures of aqueous CH_3COOH solutions of poly(Cys[CamPrOH]) were measured at 25°C by a Knauer High Speed Membrane Osmometer and recorded on a National Two-pen Recorder VP-653A. A Sartorius membrane filter, SM11536 (50 mm ϕ), was used after being conditioned in the following way. The membrane kept in 2-propanol was soaked in degassed water and placed in a vacuum dessicator, and the dessicator was evacuated intermittently by a water aspirator. Then the soaking solvent was changed to an aqueous CH_3COOH solution of desired composition by gradually increasing the CH_3COOH content. The attainment of osmotic equilibrium between the solution and the solvent was confirmed by observing no further increase in the osmotic pressure upon exchange of the solution. This took about 30 min.

The light scattering of HFIP solution of poly(Cys[CamPrOH]) was measured at room temperature ($25 \pm 2^\circ\text{C}$) on a Chromatix Low Angle Laser Light Scattering Photometer KMX-6.³ The light source was a 2 mW He-Ne Laser at 633 nm, and vertically polarized light was incident to the solution in the sample cell. The photometer is designed in such a way that the photomultiplier measures all the light scattered in the direction of a circular interval, which is defined by conical surfaces having definite apex angles for a selected scattering angle.

The light scattered at a low scattering angle is focused by a relay lens on a field stop, after passing through a circular annulus. The annulus permits to pass the light scattered in the direction of the circular interval defined by the selected scattering angle, and the field stop determines the effective length of scattering volume. A $6-7^\circ$ annulus was chosen for the solutions, so as to fix the scattering angle at 4.5° , and the field stop was set at 0.2 mm.

The scattered light passing through different parts of the annulus behaves as if the scattering has occurred for differently polarized incident light, even when the incident laser is vertically polarized: the light that has passed through the 12—6 o'clock part of the annulus is the scattered light for vertically polarized incident light, and the light that has passed through the 3—9 o'clock part of the annulus is the scattered light for horizontally polarized incident light, and so on. In sum, the light scattered at a given scattering angle for unpolarized light is collected by the field stop and its intensity is measured by the photomultiplier.

Intensities of light, incident and scattered, were recorded on a Hitachi Recorder 056. A solution or solvent was slowly sent from a syringe to the sample cell by means of a Sage Syringe Pump 341. Between the syringe and the cell was placed a membrane filter FP-010 of Sumitomo Denko Co., having a pore size of 0.1 μm .

The reduced intensity of scattered light for pure HFIP was found to be $R_0^\circ = r^2 i_0 / 2I_0 = 1.28 \times 10^{-6} \text{ cm}^{-1}$, where i_0 is the intensity of the scattered light at a distance, r , and I_0 is the intensity of incident light.

The specific refractive index increment for poly-(Cys[CamPrOH]), TD-6123, in HFIP was measured on a Brice-type differential refractometer at $25 \pm 0.01^\circ\text{C}$ over the concentration range from 0.5 to 1.5 g dl $^{-1}$. The light source was a 0.5 mW He-Ne laser, 155 of Spectra Physics Co., and its intensity was attenuated to 1% by a neutral filter of Hoya Glass Co. The deflection of the slit image focused on a filar microscope was observed by naked eye. The apparatus was calibrated with aqueous NaCl solution whose refractive indices were obtained by extrapolation of the published data⁴ to 633 nm.

The relative viscosities of DCA solutions of poly(Cys[CamPrOH]) were measured in an Ubbelohde-type capillary viscometer having a flow time of about 156 s for the solvent and capable of more than fivefold dilution. The temperature of measurement was kept at $25 \pm 0.01^\circ\text{C}$.

The circular dichroism of poly(Cys[CamPrOH]) dissolved in HFIP, H_2O , and D_2O was measured on a Jasco Circular Dichrometer J-40A in the wavelength region from 260 to 185 nm, using a jacketed cell having a path length of 1 mm. The concentration of solutions was about 0.02 g dl $^{-1}$ or 10^{-3} M in residue. The temperature was controlled by cir-

culating water of constant temperature through the jacket and read by a Takara Thermister Thermometer inserted in the cell jacket. Most experiments were carried out at $25 \pm 0.01^\circ\text{C}$, and additional experiments for determining the temperature dependence were performed at 2.5 and 70°C .

The optical rotatory dispersion of a DCA solution of poly(Cys[CamPrOH]) was recorded on a Jasco Photoelectric Polarimeter J-20 from 600 to 280 nm at room temperature ($25 \pm 2^\circ\text{C}$), using a 2 cm cell. The concentration was 0.1 g dl^{-1} .

MOLECULAR WEIGHT AND AGGREGATION IN SOLUTION

Osmotic Pressure of Aqueous CH_3COOH Solutions

Table I summarizes the solubility of poly(Cys[CamPrOH]) samples in water at room temperature and the results of osmotic pressure measurements on aqueous CH_3COOH solutions of these samples. The samples were not necessarily soluble in water at room temperature, and a minimum amount of CH_3COOH had to be added to prepare solutions of concentrations sufficiently high for the osmotic pressure measurements. Actually, the osmotic pressures of poly(Cys[CamPrOH]) in $\text{CH}_3\text{COOH-H}_2\text{O}$ were determined at 0.1, 1.0, and 5.0 vol% CH_3COOH . Figure 1 illustrates the reduced osmotic pressure, Π/c , of a sample TG-7011 in aqueous CH_3COOH as a function of concentration, c , where Π is the osmotic pressure. The values of Π/c at a given concentration decrease with increasing CH_3COOH content. A similar effect of

CH_3COOH content on the osmotic pressure was observed for all samples of poly(Cys[CamPrOH]).

The osmotic pressure gave linear plots such as shown in Figure 1. The values of the number-average molecular weight, M_n , derived from the reciprocal intercepts are listed in Table I, together with those of the second virial coefficient, B , in 0.1% CH_3COOH solutions.

The molecular weight is lowest in 0.1% CH_3COOH solution and increases with increasing CH_3COOH content. Thus we may take the values at 0.1% CH_3COOH to be the number-average molecular weights of the samples. Since the solubility or

Figure 1. Reduced osmotic pressure of TG-7011 in $\text{CH}_3\text{COOH-H}_2\text{O}$ at 25°C as a function of concentration. CH_3COOH content (vol%): $\circ$, 0.1; $\bullet$, 1.0; $\bullet$, 5.0.

Table I. Osmotic pressure and number-average molecular weight of poly(Cys[CamPrOH]) in $\text{CH}_3\text{COOH-H}_2\text{O}$ solutions of different CH_3COOH contents (vol%) (25°C)

Sample code	Solubility in H_2O or D_2O at room temperature	0.1% CH_3COOH		1.0% CH_3COOH	5.0% CH_3COOH
		M_n	B	M_n	M_n
			$10^{-5} \text{ cm}^3 \text{ g}^{-1}$		
A TG-7011	Insoluble	22,400	0.8 ₇	44,100	64,000
B TB-7041	Sparingly soluble	21,600	10.8	42,700	—
C TB-7042	Soluble	20,100	2.2 ₉	40,000	42,700
D TD-6123	Insoluble	21,100	2.7 ₀	32,000	—
E TB-6122	Soluble	15,200	0.0 ₈	27,800	21,300
F TX-4071	Soluble	11,800	2.3 ₁	21,700	23,000
G TE-6124	Easily soluble	9,600	1.7 ₇	13,300	27,400

the rate of dissolution would depend on the state of sample, especially on its moisture content,² we may conclude that the solubility of poly(Cys[CamPrOH]) in H₂O or D₂O decreases with increasing molecular weight.

Poly(Cys[CamPrOH]) associates with one another more strongly in solutions containing CH₃COOH more than 0.1%. As we have shown in previous work,² a low-molecular-weight sample TX-4071 is almost randomly coiled but has an ordered conformation to a small extent in H₂O. The addition of a small amount of CH₃COOH disrupts

this ordered conformation and causes the polymer to take on a perfect random coil. This was evidenced by the lowering of the Moffitt-Yang dispersion parameter, a_0 , and the intrinsic viscosity, $[\eta]$. Further addition of CH₃COOH up to about 90% does not alter values of these parameters very much but retains the polymer in a random coil conformation. The discrepancy between the results from the present osmotic measurement and those from the previous viscosity measurement could be reconciled if the random coil molecules of poly(Cys[CamPrOH]) associate together through some side chain interaction. The osmotic pressure is a direct measure of aggregation, while the intrinsic viscosity depends on the aggregated state, *i.e.*, on both size and shape of the aggregates. Furthermore, the Moffitt-Yang parameter, a_0 , is influenced by the environmental or solvent effect on peptide chromophores as well as by the polypeptide conformation.⁵⁻⁷

Light Scattering from HFIP Solution

The refractive index increment of poly(Cys[CamPrOH]) in HFIP measured on a sample, TD-6123, was found to be $0.227 \pm 0.005 \text{ cm}^3 \text{ g}^{-1}$. This is a rather high value, which is due to the low refractive index of HFIP. The refractive index of HFIP was assumed to be 1.274 for the He-Ne line, on the basis of the data that it is 1.2752 for the D-line⁸ and that the dispersion of aliphatic alcohols give values at 633 nm about 0.001 lower than those at 589 nm.

Figure 2 shows $Kc/\Delta R_0$ vs. c plots for four

Figure 2. Light scattering of poly(Cys[CamPrOH]) in HFIP at 25°C. Sample code: A, TG-7011; B, TB-7041; D, TD-6123; E, TB-6122.

Table II. Light scattering of HFIP solutions of poly(Cys[CamPrOH]) and reduced viscosity of its DCA solutions (25°C)

Sample code	Light scattering of HFIP solutions				Reduced viscosity of DCA solutions	
	M_w	$\bar{x}_w$	B	$\frac{M_w}{M_n}$	$[\eta]$	k'
			$10^{-3} \text{ cm}^3 \text{ g}^{-1}$		$\text{dl}^{-1} \text{ g}$	
A TG-7011	22,900	105	1.0 ₉	1.02	0.31	—
B TB-7041	22,500	103	1.3 ₃	1.04		
C TB-7042	20,200	93	1.3 ₆	1.00		
D TD-6123	19,400	89	0.8 ₆	0.92	0.36	0.23
E TB-6122	18,100	83	1.2 ₅	1.19	0.34	0.02
F TX-4071		Nonlinear plot		—	0.31	0.04
G TE-6124		Nonlinear plot		—	0.32	0.05

samples in HFIP, where ΔR_0 is the excess reduced intensity of the solution over that of the solvent, and K is the optical constant. These plots are linear and have positive slopes. From the reciprocal intercepts, the values of weight-average molecular weight M_w shown in Table II are obtained. The second virial coefficient B can be determined from the slopes and its values are also given in Table II. Two samples of the lowest molecular weights, TX-4071 and TE-6124, behave anomalously and give nonlinear plots having negative initial slopes. This indicates that low-molecular-weight poly(Cys[CamPrOH]) undergoes a concentration-dependent association in HFIP: it associates at higher concentrations but is molecularly dispersed at infinite dilution. It is shown below from circular dichroism data that poly(Cys[CamPrOH]) is randomly coiled in HFIP when the concentration is about 0.02 g dl^{-1} , which is sufficiently close to infinite dilution. However, it was not always possible to extend light scattering measurements down to polymer concentration so dilute that extrapolation to infinite dilution could be made accurately.

Table II also gives the indices of polydispersity, M_w/M_n , for the samples of poly(Cys[CamPrOH]). It is seen that these samples are rather homogeneous in molecular weight.

Viscosity Behavior of DCA Solutions

Figure 3 illustrates the plots of reduced viscosity, η_{sp}/c , for DCA solutions of three samples. Table II

Figure 3. Reduced viscosity of poly(Cys[CamPrOH]) in DCA at 25°C as a function of concentration. Sample code: D, TD-6123; E, TB-6122; G, TE-6124.

includes the values of intrinsic viscosity, $[\eta]$, and the Huggins constant, k' . These values of viscosity are rather high for given molecular weights, and its dependence on molecular weight is too weak in the region of molecular weights concerned. These results can be justified when the intrinsic viscosity-molecular weight relation in Table II is compared with those for random coil polypeptides such as poly(γ -benzyl-L-glutamate) in DCA,⁹ poly(N^5 -[3-hydroxypropyl]-L-glutamine) in H_2O ,¹⁰ and sodium poly(L-glutamate) in aqueous NaCl solutions.^{11,12} It was found that the Moffitt–Yang dispersion parameters of a sample, TX-4071, in DCA are $a_0 = -365^\circ$ and $b_0 = 42^\circ$.

These results may be interpreted in terms of the aggregation of poly(Cys[CamPrOH]) in DCA, possibly due to the partial or extensive formation of the β -conformation. Previously² we have shown that poly(Cys[CamPrOH]) is in the β -conformation and associates together in $\text{CH}_3\text{COOH-H}_2\text{O}$ mixtures containing more than 90% CH_3COOH . Accordingly, we may anticipate that a similar aggregation occurs in DCA. A strong tendency of polypeptides to form the β -conformation even in a strongly interacting solvent was reported for copolymers of L-valine with L-methionine in DCA.¹³ It is also known that poly(L-methionine) and poly(L-alanine) maintain the α -helical conformation even in DCA,^{14,15} and poly(N^5 -[3-hydroxypropyl]-L-glutamine) is helical in pure CH_3COOH .¹⁰

MOLECULAR CONFORMATION IN SOLUTION

Random Coil Conformation of Poly(Cys[CamPrOH]) in HFIP

Figure 4 shows the circular dichroism of two samples, TG-7011 and TB-7042, in HFIP at 25°C . The circular dichroism exhibits two negative bands at 225 and 198 nm and has characteristics similar to that of randomly coiled poly(*S*-carboxymethyl-L-cysteine) and poly(*S*-carboxyethyl-L-cysteine) in H_2O .¹⁶ Thus poly(Cys[CamPrOH]) is randomly coiled in HFIP. However, the residue ellipticities of the two bands, $-5,000$, and $-20,500$, are more negative than those of the two ionic poly(L-cysteine) derivatives.

The same type of circular dichroism observed

Figure 4. Circular dichroism of poly(Cys[CamPrOH]) in HFIP. Sample code: TG-7011 and TB-7042.

here was reported for low-molecular-weight poly(γ -benzyl-L-glutamate) in trifluoroethanol¹⁷ and for higher monodisperse oligopeptides such as carbobenzyldodeca(γ -methyl-L-glutamate) methyl ester in trifluoroethanol¹⁸ or carbobenzyldodeca(γ -ethyl-L-glutamate) ethyl ester in trimethyl phosphate.¹⁹ It was also exhibited by 2-methoxy-[2-ethyl]₂-acetyl-nona(L-alanine) morpholine amide²⁰ and *t*-butoxycarbonyl-hepta(L-alanine, L-valine, L-isoleucine, or L-leucine) methyl ester²¹⁻²³ in HFIP.

It is known that poly(γ -methyl-L-glutamate), poly(ϵ -carbobenzyloxy-L-lysine) and certain other polypeptides are helical in HFIP,^{8,24,25} while poly(L-alanine) assumes a doubly-bonded helix.²⁶ On the other hand, poly(γ -morpholinylethyl-L-glutamate) is randomly coiled in HFIP,⁸ although its circular dichroism differs from that of random coil poly(Cys[CamPrOH]).

Conformation of Poly(Cys[CamPrOH]) in H₂O and D₂O

As can be presumed from Table I, high-molecular-weight poly(Cys[CamPrOH]) is insoluble in H₂O as well as in D₂O at room temperature. However, we have found that a sample, either high- or low-molecular-weight, should be dissolved in

Figure 5. Effect of the temperature of dissolution on the circular dichroism of TX-4071 in H₂O at 25°C. Conditions for preparing the solution: -----, dissolved at 25°C and kept at 25°C overnight; —, dissolved and kept at 70°C for 2 h and then kept at 25°C overnight.

Figure 6. Effect of the temperature of dissolution on the circular dichroism of TB-7042 in H₂O at 25°C. Condition for preparing the solution: -----, dissolved at 25°C and kept at 25°C overnight; —, dissolved and kept at 70°C for 2 h and then kept at 25°C overnight.

H₂O or D₂O at a high temperature, in order to enhance its solubility or its rate of dissolution, and that a solution once prepared in this way remains stable even after being brought to room temperature or lower. A similar effect of temperature in preparing an H₂O solution on the solubility of a nonionic polypeptide was found for low-molecular-

weight poly(L-serine).²⁷

However, for poly(Cys[CamPrOH]), especially in the case of higher molecular weight, it was found that the temperature of dissolution has some effect on the conformation. Figure 5 shows the circular dichroism of a low-molecular-weight sample, TX-4071, dissolved in H₂O at two temperatures, 25 and 70°C, and measured at 25°C after standing overnight. The circular dichroism is scarcely dependent on the temperature of dissolution, and the random coil conformation can be assigned to poly(Cys[CamPrOH]) in H₂O. However, the circular dichroism of high-molecular-weight samples in H₂O is strongly influenced by the temperature of dissolution. Figure 6 shows the circular dichroism of a sample, TB-7042, dissolved in H₂O at 25 and 70°C and measured at 25°C after standing overnight. When compared with that of a low-molecular-weight sample, this circular dichroism is more negative at 225 nm and more positive at 198 nm, indicating that some part of the polymer changes from random coil to the β -conformation. It is also evident that a higher temperature of dissolution promotes the formation of the β -conformation, giving a positive circular dichroism around 200 nm.

It was observed by infrared measurements on the solid film regenerated from a solution that poly(DL-alanine) in H₂O or HCOOH was subject to a similar partial conversion from the random coil to the β -conformation when the solution was heated over boiling water for several min or kept at room temperature for several days, especially when the concentration was high.^{28,29} It was also described²⁷ that an H₂O solution of poly(L-serine) underwent an observable time change of circular dichroism towards an equilibrium after the preparation by heating, although the equilibrium circular dichroism was that of the random coil.

In the following, we describe the results on circular dichroism of H₂O solutions of poly(Cys[CamPrOH]) prepared by dissolving the samples at 70°C and kept at that temperature for two hours. Actually, all the samples had dissolved within 5 min. The solutions were stored at 25°C overnight before measurements. The temperature dependence of circular dichroism of solutions examined at 2.5 and 70°C, each attained from 25°C and equilibrated for two hours. The circular dichroism became constant after 30 min. The same procedure

was applied for D₂O solutions of poly(Cys[CamPrOH]) as well.

Figures 7 and 8 show the circular dichroism of

Figure 7. Circular dichroism of an H₂O solution of TX-4071 at three temperatures: ----, 2.5°C; —, 25°C; - · - · -, 75°C.

Figure 8. Circular dichroism of an H₂O solution of TG-7011 at three temperatures: ----, 2.5°C; —, 25°C; - · - · -, 75°C.

Figure 9. Temperature dependence of residue ellipticity at 198 nm of poly(Cys[CamPrOH]) in H₂O. Sample code: ●, A (TG-7011); ○, C (TB-7042); △, D (TD-6123); ▲, B (TB-7041); ■, E (TB-6122); □, F (TX-4071); ▣, G (TE-6124).

H₂O solutions of low- and high-molecular-weight samples, TX-4071 and TB-7011, respectively. The low-molecular-weight sample does not alter its conformation by the change in temperature but retains its random coil conformation. This is in agreement with our previous observation.¹ However, the high-molecular-weight sample changes its conformation from the random coil to the β -conformation when the temperature is lowered. A thermally-induced β -coil transition of poly(Cys[CamPrOH]) in H₂O is similar to that observed previously in D₂O. The circular dichroism of the β -conformation of poly(Cys[CamPrOH]) in H₂O is characterized by a negative band at 227.5 nm and a positive band at 198 nm, which is in agreement with that in D₂O at a low temperature.^{1,2}

Figure 9 summarizes the temperature dependence of residue ellipticity at 198 nm of poly(Cys[CamPrOH]) in H₂O. When the molecular weight is lower than about 18,000 or the degree of polymerization is lower than about 80, no difference in circular dichroism is observed among the three polypeptide samples: poly(Cys[CamPrOH]) is randomly coiled in H₂O at any temperatures. On the other hand, for poly(Cys[CamPrOH]) having higher molecular weight, the β -coil transition is thermally

Figure 10. Circular dichroism of a D₂O solution of TG-7011 at three temperatures: ----, 2.5°C; —, 25°C; - · - · -, 75°C.

induced and the β -conformation is formed at low temperature. The two samples, TG-7011 and TB-7042, give an identical dependence of ellipticity on

Figure 11. Temperature dependence of residue ellipticity at 198 nm of poly(Cys[CamPrOH]) in D₂O. Sample code: ●, A (TG-7011); △, D (TD-6123); ▲, B (TB-7041); ■, E (TB-6122); □, F (TX-4071); ◐, G (TE-6124).

temperature. It is, therefore, considered that, with some exception, the thermally-induced β -coil transition of poly(Cys[CamPrOH]) in H₂O is independent of molecular weight if the molecular weight is higher than 20,000 or the degree of polymerization exceeds about 90. A similar effect of molecular weight was found in the β -coil transition of poly(*S*-carboxymethyl-L-cysteine) in H₂O, for which the pH-induced transition is independent of molecular weight if the degree of polymerization is higher than about 100.¹⁶ Although it should vary with the particular polypeptide species, there may be some critical molecular weight for the onset of the β -conformation of a polypeptide in H₂O, above which the circular dichroism of the β -conformation is free from the periphery or edge effect and the 'complete' β -conformation is established.

Figure 10 illustrates the circular dichroism of D₂O solutions of a sample, TG-7011, at three temperatures. The circular dichroism of all the other samples in D₂O showed similar temperature dependence. In contrast to poly(Cys[CamPrOH]) in H₂O, essentially no difference in circular dichroism can be seen between low- and high-molecular-weight samples in D₂O, which will be more clearly shown below.

The circular dichroism of poly(Cys[CamPrOH]) in D₂O is identical with that in H₂O, whenever the polymer is in the random coil or in the β -conformation. The residue ellipticity at 225 nm for the random coil in water, -5,000, is comparable with that in HFIP, while the residue ellipticity at 198 nm for the random coil in water, -11,000, is about a half that in HFIP. Further, in water the residue ellipticity at 227.5 nm for the β -conformation is about -5,000, and that of the positive band at 198 nm is about 15,000. The latter value is much lower than those in aqueous mixtures of aliphatic alcohols of the highest alcohol contents, as was previously noted for the sample TX-4071.²

Figure 11 shows the temperature dependence of the residue ellipticity at 198 nm for poly(Cys[CamPrOH]) in D₂O. Poly(Cys[CamPrOH]) is subject to the thermally-induced β -coil transition in D₂O, irrespective of the degree of polymerization.

Since poly(Cys[CamPrOH]) gives identical circular dichroism in both H₂O and D₂O, it can be concluded that the thermal β -coil transition of low-molecular-weight poly(Cys[CamPrOH]) in D₂O is induced by the environmental or solvent effect on the stability of the β -conformation relative to the

random coil, rather than by the deuteration effect. This conclusion confirms our previous inference² derived from the experiments dealing with the effect of deuteration of D₂O on the other polypeptides.^{30,31}

On the other hand, high-molecular-weight poly(Cys[CamPrOH]) undergoes a temperature-induced β -coil transition in both H₂O and D₂O. However, as can be seen from Figures 9 and 11, the transition temperature is about 10°C higher in D₂O (47°C) than in H₂O: the β -conformation is more stable in D₂O than in H₂O. This apparent stabilization of the β -conformation may also be attributed to the solvent effect of D₂O, rather than to its deuteration effect, on poly(Cys[CamPrOH]).

Thus we may conclude that for the stability of the β -conformation relative to the random coil of poly(Cys[CamPrOH]) in aqueous media a very delicate balance between hydrogen-bonding and hydrophobic interactions is responsible, and that this balance is dependent on the molecular weight of the polypeptide and is also influenced by the change in solvent media even from H₂O to D₂O.

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