A Double Solvent-Induced β -Coil Transition of Poly-(S-[(3-hydroxypropyl)carbamoylmethyl]-L-cysteine) in Aqueous Hexafluoro-2-propanol Mixtures

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(Received June 16, 1981)

ABSTRACT: Conformations of poly(S-[(3-hydroxypropyl)carbamoylmethyl]-L-cysteine) in mixtures of hexafluoro-2-propanol (HFIP) and D₂O or H₂O have been investigated by means of circular dichroism measurements at 25°C. In HFIP, the polymer takes on a random coil conformation, exhibiting negative circular dichroism in the far ultraviolet region. In D₂O, the high-molecular-weight polymer, having molecular weight higher than 20,000, is mostly in the β -conformation, but it can assume a perfect β -conformation in HFIP-D₂O mixtures containing about 13% HFIP. It can also assume the β -conformation in HFIP-H₂O mixtures of the same composition, but to a less extent. At a lower HFIP content, the polymer is almost randomly coiled. Thus a double solvent-induced β -coil transition occurs either in HFIP-D₂O or HFIP-H₂O mixtures. The complex behavior of the polymer in these solvent mixtures can be interpreted by the common property of HFIP as an aliphatic alcohol such that its addition increases the stability of β -form polypeptide and by the specific property of HFIP acting as a strong random-coil solvent for β -form polypeptide. However, in HFIP-H₂O mixtures, the low-molecular-weight polymer remains randomly coiled throughout the entire solvent composition.

KEY WORDS Poly(S-[(3-hydroxypropyl)carbamoylmethyl]-L-cysteine) / Hexafluoro-2-propanol / Deuterium Oxide / Circular Dichroism / β -Conformation / β -Coil Transition /

In a previous paper¹ we have shown that above a threshold range of molecular weight (about 20,000) or degree of polymerization (about 90) poly(S-[(3-hydroxypropyl)-carbamoylmethyl]-L-cysteine) (poly(Cys[CamPrOH])) in H₂O undergoes a thermally-induced β -coil transition. The β -conformation is formed at 2.5°C and gradually disrupts into a random coil with raising temperature. This indicates that there is a very subtle balance between hydrogen bonding and hydrophobic interaction. Consequently, the threshold degree of polymerization disappears in D₂O, and all the samples of poly(Cys[CamPrOH]) examined underwent the thermally-induced β -coil transition in D₂O.^{1,2} The β -conformation is more stable in D₂O than in H₂O. Aliphatic alcohols also increase the stability of the β -conformation of poly(Cys[CamPrOH]) when sufficiently added to the H₂O solution.³

Originally we expected that poly(Cys[Cam-

PrOH]) had a high solubility in H₂O and D₂O and could be a nonionic water-soluble polypeptide capable of forming the β -conformation in H₂O. However, the high-molecular-weight sample was insoluble in H₂O at room temperature, and in order to increase the solubility in H₂O, we had to raise the temperature of dissolution.

On the other hand, poly(Cys[CamPrOH]) is readily soluble in hexafluoro-2-propanol (HFIP), and its conformation in this solvent was found to be the random coil by measurements of circular dichroism.¹ HFIP is transparent down to the far ultraviolet region and is a suitable solvent for making circular dichroism measurement of polypeptides.⁴ It has, however, a dual nature for the conformational stability of helical polypeptides. Poly(γ -methyl-L-glutamate) and poly(ϵ -carbobenzoxy-L-lysine) are in the α -helix conformation in HFIP, but poly(γ -morpholinylethyl-L-glutamate)

is randomly coiled in this solvent. Furthermore, poly(L-alanine) takes on a doubly-bonded helix in HFIP.^{5,6}

In the present work, we investigate the conformation of poly(Cys[CamPrOH]) in HFIP-D₂O and HFIP-H₂O mixtures of different compositions by circular dichroism measurements and examine the effect of solvent composition and molecular weight on the conformation. We have found that poly(Cys[CamPrOH]) can form the β -conformation in HFIP-D₂O mixtures at an intermediate solvent composition.

EXPERIMENTAL

Materials

Three samples of poly(Cys[CamPrOH]), two of high molecular weight and one of low molecular weight, were used in the present work. These samples were the same as those previously prepared and characterized.¹ Their codes, number-average and weight-average molecular weights are given in Table I.

Water was purified by redistilling once distilled water from alkaline KMnO₄ in a glass still. Spectrargrade D₂O (99.75% pure) and HFIP were obtained from Merck and used without further purification.

Apparatus

Circular dichroism was measured on a Jasco Circular Dichrometer J-40A, using a quartz cell of path length 1 mm. The concentration of solution was about 0.02 g dl⁻¹ or 10⁻³ M in residue. The temperature was controlled by circulating water at a constant temperature of 25 ± 0.05°C through the cell jacket. The temperature was read by a Takara

Thermistor Thermometer.

Preparation of Solutions

For circular dichroism measurements, a weighed amount of a given sample was dissolved in a known amount of HFIP, and this stock solution was diluted by adding HFIP first and then H₂O or D₂O, to give a desired composition. The solvent composition is expressed by the HFIP content or the weight fraction of HFIP (%).

RESULTS

Table I gives the values of residue ellipticity of circular dichroism in HFIP solutions. In HFIP-D₂O mixtures, poly(Cys[CamPrOH]) is subject to deuterium exchange, but its effect on the conformation of poly(Cys[CamPrOH]) was shown to be negligible.² Furthermore, it was concluded previously³ that the main influence of D₂O on polypeptide conformation was the environmental effect of D₂O. In HFIP-D₂O mixtures, the deuterium exchange between HFIP and D₂O should also occur, but this effect probably does not affect the results obtained here for the reason given below.

Figure 1 shows the circular dichroism of a high-molecular-weight sample TG-7011 in HFIP-D₂O mixtures. In HFIP, this sample has two negative bands at 227 and 198 nm, and the values of residue ellipticity of these bands are given in Table I. The sample takes on a random coil conformation in HFIP, as stated previously.¹

Until the HFIP content decreases to about 20%, the circular dichroism does not change appreciably, but below about 20% it changes drastically. The negative band at 227 nm becomes slightly deeper,

Table I. Number-average and weight-average molecular weights of poly(Cys[CamPrOH]) and circular dichroism in HFIP solution

Sample code	M_n	M_w	λ nm	$[\theta]$	λ nm	$[\theta]$
				degree cm ² decimol ⁻¹		degree cm ² decimol ⁻¹
TG-7011	22,400	22,900	198	-20,400	226	-5,100
TB-7042	20,100	20,200	197	-20,200	227	-5,600
TB-6122	15,200	18,100	197	-20,600	226	-5,400

Figure 1. Circular dichroism of TG-7011 in HFIP- D_2O mixtures. HFIP content (%): ·····, 100; ----, 23.4; —, 13.0; - - - - , 10.4; — · — · —, 3.5.

and the sign of the band at 198 nm changes from negative to positive.

At 13.0% HFIP, the negative band is the deepest and the positive band is the highest. The values of residue ellipticity are given in Table II. The positive ellipticity at 200 nm has a value as large as those found in ethanol- H_2O and propanol- H_2O mixtures previously.³ The conformation of poly(Cys[CamPrOH]) at this intermediate composition can be assigned to the β -conformation.

A further decrease in HFIP content leads to a decrease in magnitude of residue ellipticity, but at the lowest HFIP content, the positive band still

persists at 196 nm and a shallow negative band remains at 226 nm.

From these observations we find that the conformation of poly(Cys[CamPrOH]) in HFIP- D_2O mixture changes from the random coil to the β -conformation as the HFIP content drops below 20%, and that the β -conformation is partially disrupted as the HFIP content decreases to less than 10%.

The mixing of HFIP with D_2O should cause the deuterium exchange between these two solvent components, but this exchange probably does not affect the conformational change of poly(Cys[CamPrOH]) in HFIP- D_2O mixtures, since HFIP disrupts the β -conformation while D_2O tends to stabilize the β -conformation. As will be shown below, the solvent composition at which the β -conformation becomes the most stable is independent of whether one component is H_2O or D_2O .

Figure 2 shows the circular dichroism of the sample TG-7011 in HFIP- H_2O mixtures. With decreasing HFIP, the circular dichroism remains almost unchanged down to 20% HFIP. Below 20% HFIP, the circular dichroism changes as in HFIP- D_2O mixtures, and the sign of the band at 200 nm is reversed. At the optimum solvent composition, *i.e.*, at 13.5% HFIP, the β -conformation is formed. However, the positive residue ellipticity of the band at 201 nm is not as large as in HFIP- D_2O mixtures, as can be seen from Table II.

A further decrease in HFIP content reduces the magnitude of the ellipticity at 200 nm and changes its sign from positive to negative. At these HFIP contents the random coil conformation is formed. Since the sample TG-7011 was insoluble in water at 25°C, it was not possible to measure the circular dichroism in pure D_2O or H_2O .

Figure 3 shows the residue ellipticity at 200 nm as a function of HFIP content. The conformational change of high-molecular-weight samples of poly(Cys[CamPrOH]) in this solvent can be clearly seen. The other high-molecular-weight sample TB-7042 behaves similarly in HFIP- D_2O mixtures, and probably also in HFIP- H_2O mixtures. This sample was soluble in H_2O at 25°C, and its residue ellipticity at 200 nm in this solvent is also included in Figure 3. It may be considered that the β -conformation is more stable in D_2O and H_2O than in aqueous mixtures with a small amount of HFIP.

High-molecular-weight samples of poly(Cys-

Table II. Circular dichroism of poly(Cys[CamPrOH]) in HFIP-D₂O and HFIP-H₂O mixtures

Sample code	Weight fraction of HFIP %	λ nm	$[\theta]$ degree cm ⁻² decimol ⁻¹	λ nm	$[\theta]$ degree cm ⁻² decimol ⁻¹
TG-7011	(D ₂ O)	200	34,000	227.5	-9,000
	(H ₂ O)	201	10,000	227.5	-5,800
TB-7042	(D ₂ O)	201	28,000	227.5	-7,400
	(H ₂ O)				
TB-6122	(D ₂ O)	200	10,500	227.5	-5,400
	(H ₂ O)	200	-12,000	220	-4,300

Figure 2. Circular dichroism of TG-7011 in HFIP-H₂O mixtures. HFIP content (%): ·····, 100; ----, 15.0; —, 11.5; - · - ·, 3.9.

[CamPrOH]) behave similarly in the solvent-induced β -coil transition in HFIP-D₂O or HFIP-H₂O mixtures, and thus the conformational stability is independent of molecular weight, provided that the molecular weight is higher than the threshold value of about 20,000.

Figure 3. Residue ellipticity at 200 nm as a function of solvent composition: Circles, TG-7011; squares, TB-7042; open symbols, D₂O; filled symbols, H₂O.

On the other hand, the low-molecular-weight sample behaves differently. Figure 4 shows the circular dichroism of the low-molecular-weight sample TB-6122 in HFIP-D₂O mixtures. The circular dichroism changes with HFIP content and behaves somewhat similarly to that of the high-molecular-weight samples in HFIP-H₂O mixtures. At the

Figure 4. Circular dichroism of TB-6122 in HFIP- D_2O mixtures. HFIP content (%): ·····, 100; ----, 18.6; —, 13.7; — · —, 3.6.

Figure 5. Circular dichroism of TB-6122 in HFIP- H_2O mixtures. HFIP content (%): ·····, 100; ----, 15.0; —, 13.5; — · —, 4.0.

intermediate solvent composition, *i.e.*, at 13.7% HFIP content, the magnitudes of residue ellipticity of the two bands at 200 and 226 nm become the largest. Table II gives the values of residue ellipticity of these bands at the optimum solvent composition for the formation of β -conformation. Thus, even the low-molecular-weight sample of poly(Cys[CamPrOH]) undergoes a similar conformational change in HFIP- D_2O mixtures, as observed with the high-molecular-weight samples in HFIP- D_2O and HFIP- H_2O mixtures.

Figure 5 shows the circular dichroism of the sample TB-6122 in HFIP- H_2O mixtures. This shows that no conformational change occurs but only a small perturbation exists around 13% HFIP content. Figure 6 shows the residue ellipticity at 200 nm as a function of HFIP content for the low-molecular-weight sample TB-6122. It can be seen that the conformational change occurs in HFIP- D_2O mixtures but not in HFIP- H_2O mixtures.

Figure 6. Residue ellipticity at 200 nm as a function of solvent composition for TB-6122: ○, D_2O ; ●, H_2O .

DISCUSSION

In a previous work,³ we have observed that the addition of a small amount of aliphatic alcohol to an H₂O solution of a low-molecular-weight sample of poly(Cys[CamPrOH]) reduces the residue ellipticity at 200 nm. This was indicative of the disruption of a small amount of β -conformation remaining in H₂O. The same can be expected to occur even with high-molecular-weight samples, if they were soluble in H₂O or D₂O. Since HFIP is an alcohol, we can expect the same action for HFIP to disrupt the β -conformation remaining in H₂O or D₂O and to change the polymer to the state of a random coil. Thus, at low HFIP contents, poly(Cys[CamPrOH]) in HFIP-D₂O mixtures is randomly coiled, as in aqueous alcoholic mixtures.

With increasing HFIP content, poly(Cys[CamPrOH]) undergoes a coil-to- β transition, quite sharply at HFIP contents higher than about 10%. The β -conformation is the most stable at a 13% HFIP content.

In aqueous aliphatic alcohol mixtures, poly(Cys[CamPrOH]) becomes insoluble above the highest alcohol content at which the β -conformation is most completely formed. In HFIP-D₂O mixtures as well as in HFIP-H₂O mixtures the situation is considerably different, since HFIP has a strong solvent power to dissolve poly(Cys[CamPrOH]).

Isobaric vapor-liquid phase diagram⁶ and vapor pressure measurements⁷ indicate that, with increasing HFIP content, the activity of HFIP in HFIP-H₂O mixtures increases very rapidly at low HFIP contents, but almost ceases to increase at HFIP contents ranging from 13 to 70%, or at the mole fractions from 0.01 to 0.20. This means that a strong repulsive interaction is operative between HFIP and H₂O or D₂O; the repulsion almost leads to a liquid-liquid phase separation. At HFIP contents higher than 13% the solvent mixture behaves as if it were separated into two phases, one being richer and the other poorer in HFIP.

The composition of HFIP-H₂O or HFIP-D₂O mixtures, above which the activity of HFIP becomes nearly independent of solvent composition, would not be influenced by the presence of a small amount of dissolved poly(Cys[CamPrOH]). In view of the pseudo two-phase character of the solvent, poly(Cys[CamPrOH]) is in a state analogous to

being dissolved in an HFIP-rich phase and dispersed in an HFIP-poor phase, when the HFIP content of solvent mixture exceeds 13%, since poly(Cys[CamPrOH]) is more soluble in HFIP than in H₂O or D₂O. Thus, poly(Cys[CamPrOH]) highly solvated by HFIP can still be dissolved in the solvent mixture. The conformation of poly(Cys[CamPrOH]) is then the random coil, as in pure HFIP.

We summarize the conformational transitions of poly(Cys[CamPrOH]) in HFIP-D₂O or HFIP-H₂O mixtures as follows:

In contrast, the low-molecular-weight poly(Cys[CamPrOH]) in HFIP-H₂O mixtures remains in the random coil throughout entire range of solvent composition.

In general, we may consider that an aliphatic alcohol has a dual effect on the conformational stability of polypeptide capable of forming β -conformation on being added to an H₂O or D₂O solution. The addition of such an alcohol at the most dilute concentration to an aqueous solution of polypeptide disrupts the β -conformation remaining in H₂O or D₂O in a trace amount and stabilizes the random coil. At the intermediate content, however, the alcohol causes the polypeptide conformation to change from the random coil to the β -conformation. In addition to these common properties of alcohol, we can note that HFIP displays specific properties in aqueous mixtures, such as stated above,^{6,7} caused by the repulsive interaction against H₂O or D₂O, and also such as surface activity.⁸

It was reported that poly(L-alanine) showed characteristic circular dichroism in HFIP.^{5,9} This type of circular dichroism was assigned to a conformation designated as the doubly-bonded helix,⁵ in which an additional hydrogen bond links the amide carbonyl group with HFIP, besides the intra-molecular hydrogen bond. On the other hand, poly(L-alanine) in HFIP-H₂O mixture at 18% HFIP or at a mole fraction of 0.3 HFIP is in the α -helix. Such a conformational change probably results from the particular solvent properties of HFIP-H₂O mixtures.

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