The Influence of Serine Residue on the Poly(Ser-co-Lys) in HFIP / Water Solutions

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The conformational transition of poly(Ser:22-co-Lys:78) was studied in a mixed solution of 1,1,1,3,3,3,-hexafluoroisopropanol (HFIP) and water by circular dichroism (CD) spectroscopy. The structure of this polymer was α-helix in 100% HFIP solution and random coil in 100% water. The conformational transition was observed in 50 / 50 (HFIP : water).

It is well known that serine is central to the action of the enzyme called serine protease, such as thrombin and chymotrypsin. The structure and active mechanisms of these enzymes have been closely investigated.[1-4] Only recently it was found that high molecular weight and water-soluble poly(Ser:22-co-Lys:78) has higher penetrability into cells than poly(Lys), and has been used to make progress in the development of materials for drug delivery systems (DDS).[5-9]

In this letter, we address our remarks to the serine residue, and report on the behavior of poly(Ser:22-co-Lys:78) synthesized using NCAs in HFIP / water solutions. Since the cell membranes are hydrophobic and body fluid is hydrophilic, it is necessary to know the conformational transition of the polymer in variable percentages of lipid / water for medicinal purposes. Besides, it seems necessary to gather quickly basic data on the behavior of serine residue in the copolymer as well, since little has been reported to date on the related serine residue

Quadriefoglio, et al. have determined the circular dichroism (CD) spectra of a low molecular weight poly-L-serine dissolved in water and in water-organic solvent mixtures. The CD spectrum of this sample dissolved in water is β-conformation, and in 8M LiCl it is random-coil.[10]

Table 1. Polypeptides used in the experiment

No.	Polypeptides	Component	$[\eta]$	M[11]
1.	poly(Lys)		4.20[a]	550000
2.	poly(Ser,Lys)	Ser/Lys : 12/88	0.50[b]	103600
3.	Poly(Ala,Lys)	Ala/Lys : 20/80	0.40[a]	86800
4.	Poly(Ser,Lys)	Ser/Lys : 22/78	0.47[b]	98700
5.	Poly(Ser,Lys)	Ser/Lys : 28/72	—	—
6.	Poly(Ser,Lys)	Ser/Lys : 40/60	—	—

a : Ubbelode-, b : Ostwald viscometer c=0.45g/dl.

Table 1. shows the property of poly(Ser:22-co-Lys:78) and the copolypeptides used for comparison in this study. They have a molecular weight sufficient to act on polymers, and the polymers of No. 1.-5. were soluble in HFIP and water at pH around neutrality. No. 6. was insoluble in both HFIP and water. The components were procured by ^{1}H NMR. The ellipticity $|\theta|$ of the CD spectra were recorded by a previous method.[12]

The CD spectra of the polymers of No. 1.-5. had a minimum peak at 196-198 nm in an aqueous solution, which correspond to a random coil structure (Figure 1.). On the other hand, they had two negative peaks at 208-206 nm and 216-224 nm, characteristic of an α-helix structure in HFIP (Figure 2 .).

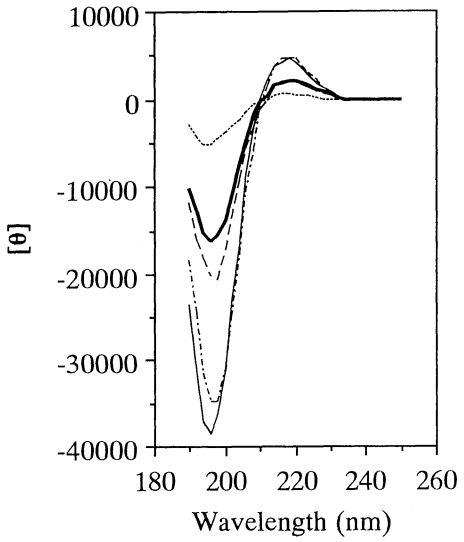

Figure 1. CD spectra in water solution: ——, poly(Lys); – – –, poly(Ser:12,Lys:88); – – –, poly(Ala:20,Lys:80); ——, poly(Ser:22,Lys:78); ·····, poly(Ser:28,Lys:72).

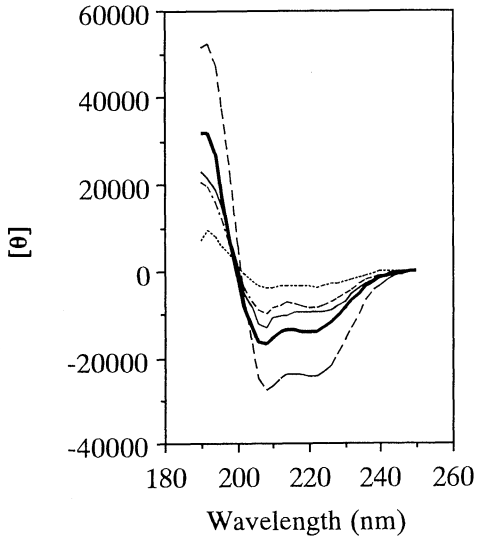

Figure 2. CD spectra in HFIP solution: ——, poly(Lys); – – –, poly(Ser:12,Lys:88); – – –, poly(Ala:20,Lys:80); ——, poly(Ser:22,Lys:78); ·····, poly(Ser:28,Lys:72).

The conformational transition from α-helix to random coil of poly(Ser:22-co-Lys:78) started in 50% water, however the conformation remained helical (Figure 3.). But the polypeptides of No. 1, 2, 3 and 5 were perfectly random coil in HFIP : water = 50 : 50 (Figure 3.). As for the homo-polymer of lysine, the conformation of this polymer changed to a random coil because the amino group of lysine residue was strongly affected by water. The conformation of poly(Ser:12-co-Lys:88), which had a lower content of serine than the former poly(Ser:22,Lys:78), was dependent on the lysine residue. The conformation of poly(Ala:20-co-Lys:80), containing a substitute alanine residue having a CH_3 side chain, was not influenced by the alanine residue, and the conformation was about the same as the conformation of poly(Lys) because the methyl group of alanine has no affinity for water and has a weak intramolecular hydrophobic bond in water solution. Poly(Ser:28-co-Lys:72) was gelled by the strongly hydrophilic hydroxyl group of serine residue in water and it was difficult to dissolve it in HFIP because of a strong intermolecular hydrogen bond of the hydroxyl group of serine. In contrast, the hydroxyl group of serine had a weak intramolecular hydrogen bond. Given these conditions, the intramolecular hydrogen bond forming an α-helix in the peptide main chain was destroyed in HFIP / water = 50 : 50, and the conformation was changed to a random structure (Figure 4. a). Poly(Ser:40-co-Lys:60) which had a larger serine residue, was nearly insoluble in almost any solution because it had many intermolecular hydrogen bonds for the hydroxyl group of serine.

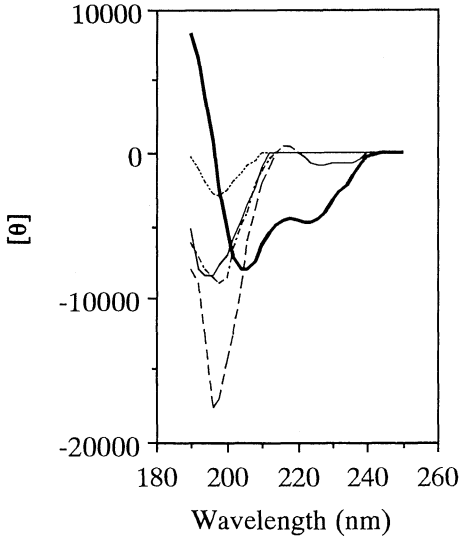

Figure 3. CD specra in HFIP / water = 50 / 50 solution: ——, poly(Lys); – – –, poly(Ser:12,Lys:88); – – –, poly(Ala:20,Lys:80); ——, poly(Ser:22,Lys:78); ·····, poly(Ser:28,Lys:72).

We propose the following concerning the high stability of the helix structure with poly(Ser:22,Lys:78) in HFIP / water = 50 : 50 solution. The polymer had a larger number of intramolecular hydrogen bonds for the serine residue side chain than intermolecular hydrogen bonds (Figure 4. b). The α-helix of this polymer was stabilized by two kinds of intramolecular hydrogen bonds (Figure 4. c). The delicate proportion of serine residue (having hydroxyl group side chain) in the polymer influenced the formation of intramolecular and intermolecular hydrogen bonds.

The intramolecular hydrogen bond in the serine residue side chain affected the conformational transition in HFIP / water solution.

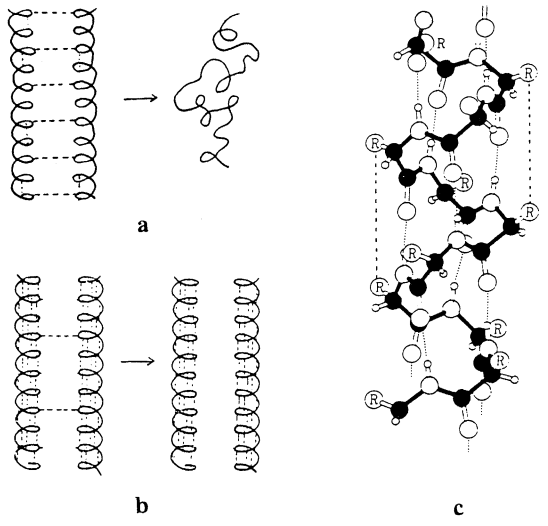

Figure 4. The illustrations of intermolecular and intramolecular hydrogen bonds of the hydroxyl group of side chain : **a** and **b**; and α-helix **c**.

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