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Secondary Structure of Poly-L-arginine and Its Derivatives*1

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The conformations of poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine, poly-N^{ω}-carbobenzyloxy-L-arginine, and poly-L-arginine hydrobromide were studied by means of their infrared spectra (IR), X-ray photographs, optical rotatory dispersions (ORD), and circular dichroisms (CD). The presence of the α-helical conformation in poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine and poly-N^{ω}-carbobenzyloxy-L-arginine was confirmed by X-ray photographs and by their IR spectra. The ORD and the CD curves of these polymers were found to be characteristic of the α-helical conformation. It was also found that these polypeptides have a random coil conformation in trifluoroacetic acid. In the (2-chloroethanol : trifluoroacetic acid) solvent system, the typical helix-coil transition of poly-N^{ω}-carbobenzyloxy-L-arginine was observed at 20—30% trifluoroacetic acid. The ORD and the CD curves of poly-L-arginine showed that the polymer took a random coil structure in the pH range from 2.7 to 11.0. However, when the pH is increased from 11.0 to 11.5, the spectra gradually change into the α-helix structure. Moreover, the polypeptide predominantly took the α-helix structure at pH 12.5. From the ORD and CD data, the polypeptide can be considered to have a right-handed α-helix conformation.

Considerable interest has recently been shown in the secondary structure of many synthetic polypeptides and proteins in the solid state and in solution. The protamins are strongly basic proteins which have high arginine contents, and their con-

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formations have been found by the NMR[1] and the ORD[2,3] measurements to be almost random coil structures in aqueous solutions. However, the physicochemical properties of poly-L-arginine and its derivatives have not been reported. Recently we have prepared poly-L-arginine as an useful model of the protamins and have also prepared, by the NCA method,[4] two kinds of protected polyarginine as intermediates. In this paper, the physical properties and the secondary structure of poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine, poly-N^{ω}-carbobenzyloxy-L-arginine, and poly-L-arginine hydrobromide in the solid state and in solution will be discussed using the date obtained from the IR spectra, the X-ray analyses, and the ORD and CD.

Experimenta

Materials. Poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine (I), poly-N^{ω}-carbobenzyloxy-L-arginine (II), and poly-L-arginine hydrobromide (III) were prepared by the NCA method described in a previous paper.[4] The degree of polymerization of these polypeptides is found to be *ca.* 70 by carboxyl end-group analysis.

Measurements. The IR spectra were recorded by the use of the potassium bromide-pellet technique on a Jasco IR spectrophotometer, Model DS-301. The X-ray diffraction photographs were taken with a Rigaku-denki Geigerflex, using a Cu-target. The ORD and CD were measured on a Jasco Model ORD/UV-5 ORD recorder with a CD attachment at room temperature, using the cells with light paths of between 0.1 and 100 mm. The aqueous solutions of poly-L-arginine for the ORD and CD measurements were prepared by dissolving poly-L-arginine hydrobromide in water and by adjusting the pH with 0.1N NaOH.

Results and Discussions

IR Studies and X-Ray Analyses. The IR absorption spectra of I, II, and III showed absorptions at 1655 cm^{-1} (Amide I) and 1545 cm^{-1} (Amide II), suggesting that the polymer could have the α-structure (Fig. 1). The absorption shoulder at 1545 cm^{-1} and the other absorptions at 1515cm^{-1}, 1615 cm^{-1} and 1725 cm^{-1} of the polymers I and II may be due to the influence of the protected guanido group in polyarginine. The spectrum of III is simplified in all regions because of the removal

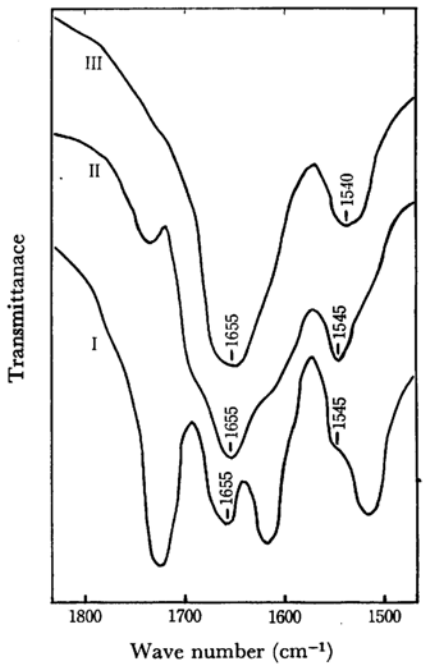

Fig. 1. IR spectra of poly-L-arginine hydrobromide and derivatives.
I: poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine;
II: poly-N^{ω}-carbobenzyloxy-L-arginine;
III: poly-L-arginine hydrobromide

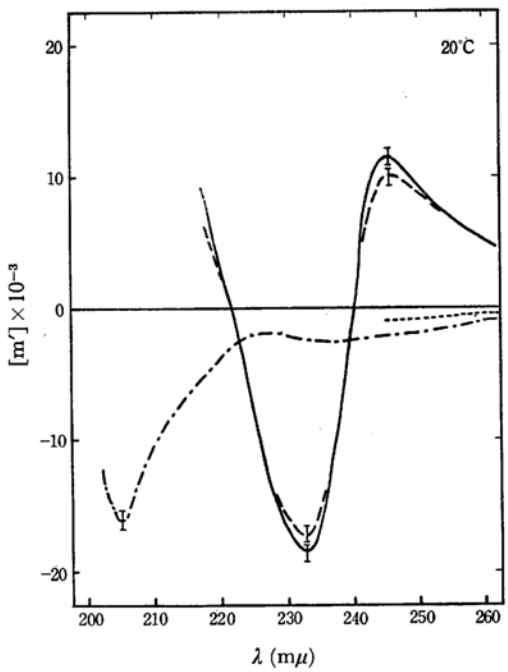

Fig. 2. ORD of poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine in THF (——), in dioxane (– – –), in TFA (- - - -) and in 2-chloroethanol (– · –).

1) E. M. Bradbury, C. Crane-Robinson, H. W. E. Rattle and R. M. Stephans, "Conformation of Biopolymers," ed. G. N. Ramachandam, Academic Press, London and New York (1967), p. 583.

2) B. Jirgensons and L. S. Hnilica, *Biochim. Biophys. Acta*, **109**, 241 (1965).

3) E. M. Bradbury, C. Crane-Robinson, H. Goldman, H. W. E. Rattle and R. M. Stephans, *J. Mol. Biol.*, **29**, 507 (1967).

4) T. Hayakawa, Y. Kondo, H. Yamamoto and Y. Murakami, This Bulletin, **42**, 479 (1969).

of the protecting group of the guanido group and showed absorptions at 1655 cm^{-1} and 1540 cm^{-1}. X-ray analyses of the polymers I, II, and III were also carried out. The X-ray diffraction patterns of I and II indicated the presence of a 18 Å reflection. Similarly, the X-ray photograph of poly-γ-benzyl-L-glutamate[5] in the α-helix structure shows the presence of a 13 Å reflection. This could be interpreted as indicating that a helix-helix interval of an α-helical polypeptide is broadened from 13 Å to 18 Å for poly-L-arginine derivatives (I and II) because of the bulky side chain. Poly-L-arginine hydrobromide (III) gives an almost amorphous photograph.

Optical Rotatory Dispersion and Circular Dichroism. *Poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine.* The ORD curves of poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine in tetrahydrofuran (THF), in dioxane, in 2-chloroethanol, and in trifluoroacetic acid (TFA) are shown in Fig. 2. In THF and in dioxane, the polypeptide exhibits a positive Cotton effect, with a strong peak at 245—247 mμ, followed by a deep trough at 233 mμ; the crossover point lies at 240 mμ. A second positive Cotton effect can be expected below 220 mμ with the crossover point at 222 mμ, but this is not clearly evident from the ORD curves. The CD curves of the polypeptide in THF and in dioxane are recorded in order to resolve any possible overlapping of Cotton effects in this region. The CD curves are shown in Fig. 3. The CD curves of the polypeptide reveal a strongly positive dichroism centered at 235 mμ in the wavelength range 250—200 mμ. Two negative dichroism bands, at 221 mμ and 214 mμ, are observed for these solutions; a positive dichroism band can also be expected below 200 mμ. The positive dichrosim band at 235 mμ can be assigned to the π–π^* exciton transition of the benzyl group. The negative ellipticity band at 221 mμ appears to be the n–π^* peptide electronic transition associated with the α-helical conformation of polypeptide, while the negative band at 214 mμ and the positive band below 200 mμ are to be assigned to the parallel-polarized and perpendicular-polarized π–π^* exciton transitions of the peptide groups respectively.[6] These dichroism bands are found in approximately the same positions and have the same signs and magnitudes as those observed in right-handed α-helix polypeptides. From these CD and ORD measurements, this polypeptide can be considered to have a right-handed α-helix conformation in THF and in dioxane.[6] When TFA and 2-chloroethanol are used as the solvents (Fig. 2), the peak at 245 mμ disappears. The ORD and the CD curves of the polypeptide in 2-chloroethanol (Figs. 2 and 3) are characteristic of the random coil conformation; there is a 238 mμ trough, a 226 mμ peak, and a 205 mμ trough in the ORD curve, and

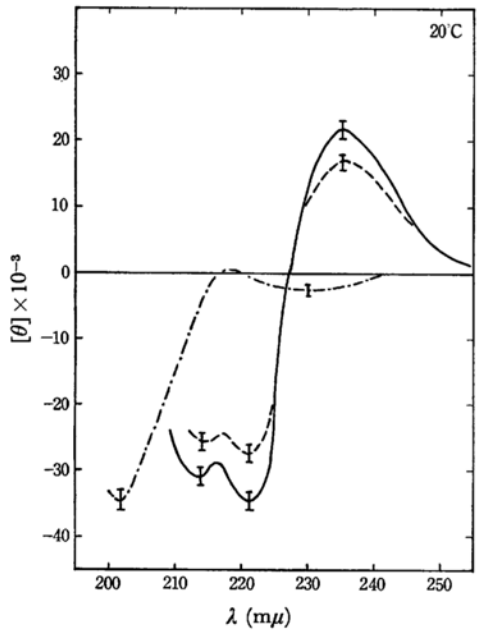

Fig. 3. CD of poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine in THF (——), in dioxane (– – –) and in 2-chloroethanol (– · –).

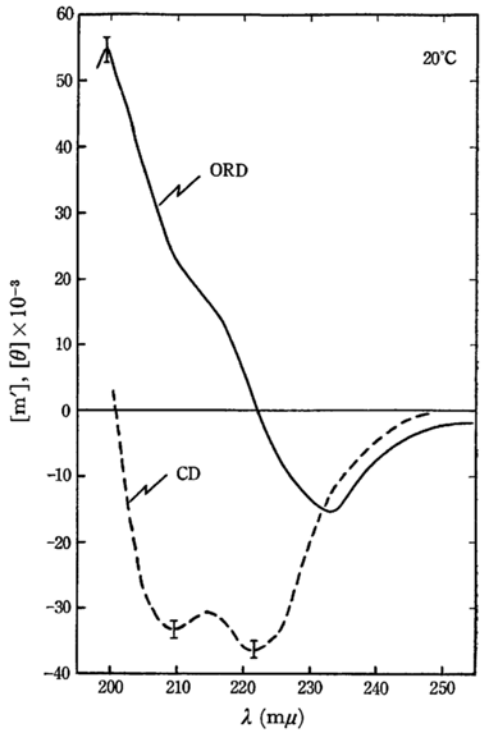

Fig. 4. ORD and CD of poly-N^{ω}-carbobenzyloxy-L-arginine in 2-chloroethanol.

5) C. H. Bamford, A. Elliot and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York (1956), p. 263; Y. Mitsui, Y. Iitaka and M. Tsuboi, *J. Mol. Biol.*, **24**, 15 (1967).

a small negative dichroism band at 230 mμ, a small positive dichroism band at 217 mμ, and a large negative dichroism band at 202 mμ in the CD curve. These results suggest that the phenyl groups near the backbone of the main chain exist in a dissymmetric environment in helix-supporting solvents (THF, dioxane). In TFA and in 2-chloroethanol, the polypeptide exists as a random coil and the dissymmetry is lost.[7]

Poly-N^{ω}-carbobenzyloxy-L-arginine. The ORD and the CD curves of poly-N^{ω}-carbobenzyloxy-L-arginine in 2-chloroethanol are shown in Fig. 4. The polymer has a 233 mμ trough and a 199 mμ peak, with a shoulder near 213 mμ, on the ORD curve; it also has two negative dichroism bands, at 222 and 210 mμ.[6] These spectra suggest that the polypeptide has a right-handed α-helix structure. The Moffitt-Yang plot for poly-N^{ω}-carbobenzyloxy-L-arginine in mixed solvents (TFA-2-chloroethanol) is shown in Fig. 5. The ORD of this polypeptide in (TFA : 2-chloroethanol) solutions fits the Moffitt-Yang equation. The coefficient of the second term, b_0, is found to be dependent on the mol% of TFA. In 100% TFA, b_0 is zero and a_0 is −310, while in 100% 2-chloroethanol b_0 is −600 and a_0 is +360.

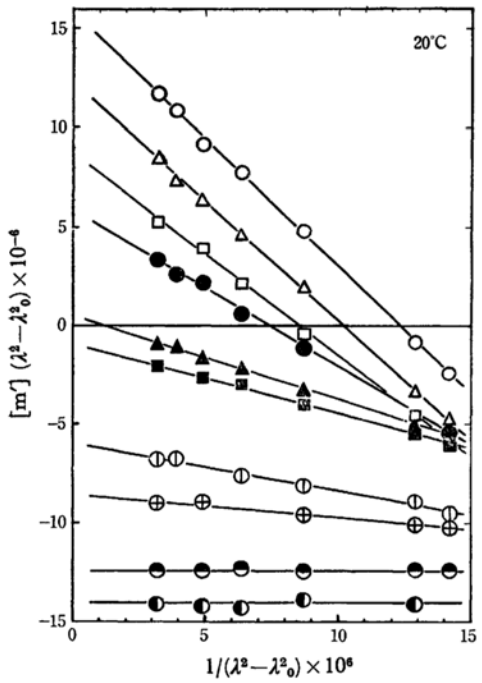

Fig. 5. Moffitt-Yang plot for poly-N^{ω}-carbobenzyloxy-L-arginine in mixed solvents (TFA-2-chloroethanol).
Volume per cent of TFA: ○, 0; △, 5; □, 10; ●, 20; ▲, 25; ■, 35; ⊕, 45; ⊕, 65; ◓, 80; ◐, 100

These results suggest that the polypeptide has a random coil structure in TFA and that it is a right-handed α-helix structure in 2-chloroethanol. More-

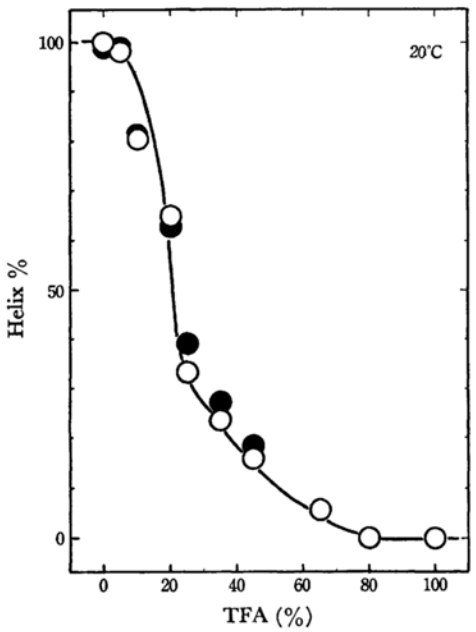

Fig. 6. Helix-coiled transition of poly-N^{ω}-carbobenzyloxy-L-arginine in TFA-2-chloroethanol mixtures.
○: from the b_0; ●: from the $[m']_{233}$

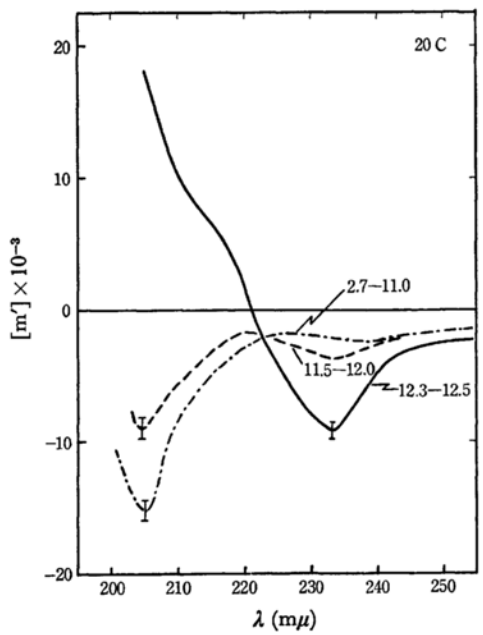

Fig. 7. ORD of poly-L-arginine as a function of pH in aqueous media.
pH 2.7—11.0 (-·-·-), pH 11.5—12.0 (------) and pH 12.3—12.5* (——).
*: water-methanol (2:1, v/v)

6) G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).

7) M. Goodman and E. Peggion, *Biochemistry*, **6**, 1533 (1967).

over, the helix-coil transition of the polypeptide in mixed solvents typically takes place at 20—30% TFA (Fig. 6). The helical contents are calculated by two methods[8,9]: a) from the b_0 value for the Moffitt-Yang plot, and b) by using the magnitude, $[m']_{233}$, in the ultraviolet-region ORD measurements.

Poly-L-arginine. The ORD curves of poly-L-arginine as a function of the pH in aqueous media are shown in Fig. 7. In the pH range 2.7—11.0, the polypeptide is in a random coil structure which has a 238 mμ trough, a 227 mμ peak, and a 205 mμ trough. However, when the pH is increased from 11.0 to 11.5, a remarkable change in the ORD spectrum is observed: the peak at 227 mμ disappears and the trough at 238 mμ shifts to 233 mμ. The solubility of the polypeptide decreases at pH values higher than 12.0. Therefore, ORD and CD measurements were carried out in methanol-water media. In the pH range 12.3—12.5, the polypeptide has predominantly the α-helix conformation: a trough with $[m']_{233} \cong -9000$, a crossover point at 221 mμ, and a shoulder near 214 mμ. However, the magnitude of the $[m']_{233}$ value for the polypeptide is smaller than the magnitude of the $[m']_{233} \cong -15000$ values of poly-L-glutamic acid (pH 4)[9] and poly-L-lysine (pH 11)[10] in aqueous solutions. When the pH is increased to 12.6, the polypeptide precipitates. It could be assumed that the coil-helix transition of the polypeptide takes place in this solution (pH 12.5), but this was not evident from the ORD spectra. The CD curves of the polypeptide as a function of the pH are shown in Fig. 8. In the pH range 2.7—11.0 the CD spectrum shows the same curve as that observed in the ORD; *i. e.*, the polypeptide shows the characteristics of a random coil, a small negative dichroism band at 230 mμ, and a small positive dichroism band at 218 mμ, while a large negative dichroism band may be anticipated below 200 mμ. In the pH range 11.5—12.0 the CD spectrum shows that the polypeptide partially takes the α-helix structure.

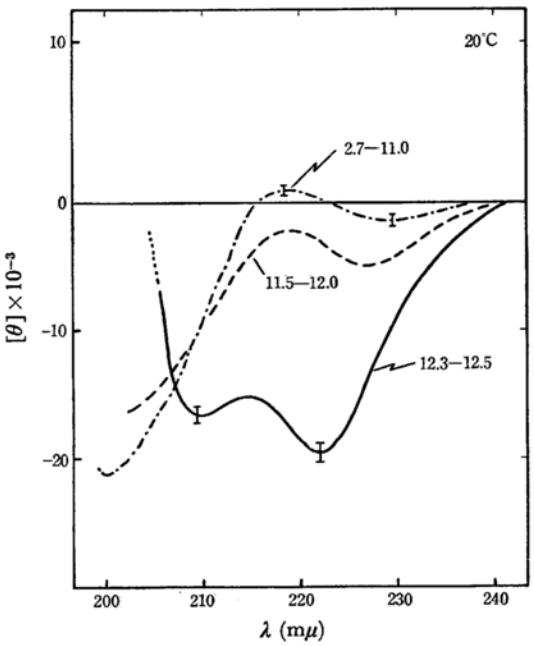

Fig. 8. CD of poly-L-arginine as a function of pH in aqueous media.
pH 2.7—11.0 (-·-·-), pH 11.5—12.0 (-----) and pH 12.3—12.5* (——).
*: water-methanol (2 : 1, v/v).

The polypeptide has predominantly the right-handed α-helix conformation between the pH values of 12.3 and 12.5: two negative dichroism bands, at 222 and 209 mμ, with $[\theta]_{222} \cong -19000$ and $[\theta]_{209} \cong -17000$ values respectively. However, the $[\theta]_{222}$ and the $[\theta]_{209}$ values are smaller than those of usual polypeptides.[6,10] These results may be assumed to indicate either that the polypeptide mingle with some random coil conformation or that the molecular weight of the polypeptide is much smaller than that of usual polypeptides.

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8) E. Iizuka and J. T. Yang, *Biochemistry*, **4**, 1249 (1965).

9) J. Y. Cassim and J. T. Yang, *Biochem. Biophys. Res. Commun.*, **26**, 58 (1967).

10) B. Davidson and G. D. Fasman, *Biochemistry*, **6**, 1616 (1967).