Side-Chain Effect on the Helix Stability of Poly-α-amino Acids

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Contribution from the Chemical Research Institute of Nonaqueous Solutions, Tohoku University, Sendai, Japan. Received August 18, 1969

Abstract: The relative stability of the helical structures of poly-α-amino acids derived from L-lysine and its lower homologs, L-ornithine and L-α,γ-diaminobutyric acid, was studied with optical rotatory dispersion, circular dichroism, and infrared absorption measurements. It was found that poly(L-α,γ-diaminobutyric acid) exists in an almost randomly coiled form in an aqueous solution even at pH values higher than 10, where poly-L-lysine and poly-L-ornithine take helical structures almost completely and partially, respectively. On the contrary, poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) was found to form a more stable helical structure than either poly-ε-N-carbobenzoxy-L-lysine or poly-δ-N-carbobenzoxy-L-ornithine in a chloroform–dichloroacetic acid solvent system. These observations are explained in terms of main chain–side chain or side chain–side chain interactions through hydrogen bonds.

M any conformational analyses of various synthetic poly-α-amino acids have been studied in order to elucidate the behavior of proteins in the living system.[1] In connection with our study[2] on poly-L-lysine–metal complexes as enzyme models, it seemed of interest to study the secondary structures of poly-α-amino acids derived from L-lysine and its lower homologs, L-ornithine and L-α,γ-diaminobutyric acid, in solution. Though there have been many studies[1] on the helix–coil transitions of poly-L-lysine and poly-ε-N-carbobenzoxy-L-lysine and some studies[3–5] on poly-L-ornithine, no conformational studies of poly-δ-N-carbobenzoxy-L-ornithine, poly(L-α,γ-diaminobutyric acid), and poly-(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) have been reported except in our preliminary communication.[6]

Results and Discussion

Poly-α-amino Acids with Amino Groups in the Side Chains. The optical rotatory dispersion (ORD) and circular dichroism (CD) measurements of poly-L-lysine, poly-L-ornithine, and poly(L-α,γ-diaminobutyric acid) were carried out in aqueous solution at various pH values at 20°. From these spectra, Moffitt's b_0,[7] reduced residue rotation at 233 mμ, $[m']_{233}$, and residue ellipticity at 222 mμ, $[\theta]_{222}$, which are related to the helix content of poly-α-amino acids, were calculated. Plots of these data as a function of pH are shown in Figures 1, 2, and 3.

From these figures, it can be seen that poly-L-lysine shows a sharp helix–coil transition at about pH 10, even when it has a relatively low degree of polymerization, and that poly-L-ornithine also shows a sharp transition at about the same pH, approaching 60% helix at pH 12.[6] On the other hand, it was found that the helix content of poly(L-α,γ-diaminobutyric acid) was near zero over the whole pH range measured, even though

the degree of polymerization of the sample used here was higher than that of the lower one of the poly-L-lysine sample used. Figure 4 includes the CD spectra of poly(L-α,γ-diaminobutyric acid) at both the neutral (pH 7.3) and alkaline (pH 11.8) solutions and the deviation of the curve at pH 11.8 from that at pH 7.3.

From the figure, poly(L-α,γ-diaminobutyric acid) seems to suffer a very slight change, from a random coil up to a few per cent of helix, with increase of the pH value of the solution. However, the possibility that the β structure exists at the higher pH cannot be completely excluded.[8] This extraordinary instability of the secondary structure of poly(L-α,γ-diaminobutyric acid) may be due to the relatively strong interaction between the amino groups in the side chains and the nearby carbonyl groups in the main chain.

In Figure 1, the results obtained from addition of salts to poly(L-α,γ-diaminobutyric acid) solution at pH 12 are also indicated. As in the case of poly-L-lysine,[9] the addition of 2.0 M KSCN to the solution induced an appreciable coil-to-helix transition, while the addition of 2.0 M KCl had little effect.

Figure 5 shows amide I bands in the infrared absorption spectra of these polymers in both neutral (pD *ca.* 7) and alkaline (pD *ca.* 11) D_2O solutions. The spectra of both samples of poly-L-lysine in the neutral D_2O solution show a strong deuterated α band between 1640 and 1645 cm^{-1} accompanied by a shoulder or σ band at 1660 cm^{-1}, due to deuterated amide I bands such as expected in random conformation.[10] This σ band disappears in the spectrum of the polymer in the alkaline D_2O solution. This spectral change seems to correspond to the conformational change of poly-L-lysine. The spectral features of poly-L-ornithine are similar to those of poly-L-lysine. On the contrary, the spectrum of poly(L-α,γ-diaminobutyric acid) shows a peak around 1655 cm^{-1} in both neutral (pD *ca.* 7) and alkaline (pD *ca.* 11) solution, which seems to reflect that little if any transformation occurs in the polymer. These observations on the infrared absorption spectra agree with the ORD and CD results.

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(6) M. Hatano, M. Yoneyama, I. Ito, T. Nozawa, and M. Nakai, *J. Amer. Chem. Soc.*, **91**, 2165 (1969).
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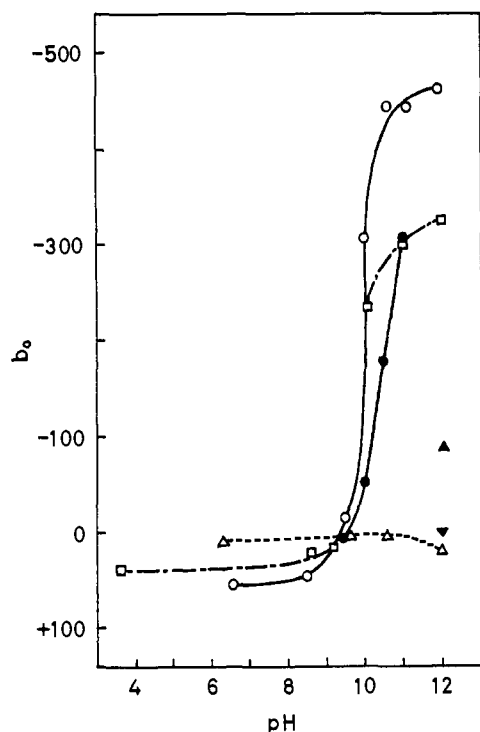(10) E. R. Blout and M. Idelson, *J. Amer. Chem. Soc.*, **80**, 4909 (1958).

Figure 1. pH dependence of Moffitt's b_0 values of poly-α-amino acids in aqueous solutions: O, poly-L-lysine (160); ●, poly-L-lysine (46); □, poly-L-ornithine (190); △, poly(L-α,γ-diaminobutyric acid) (110). The numbers in parentheses are degrees of polymerization of the parent N-carbobenzoxylated poly-α-amino acids. The b_0 values in the case of addition of salts to poly(L-α,γ-diaminobutyric acid) solution at pH 12 are also indicated: ▲, 2.0 M KSCN; ▼, 2.0 M KCl.
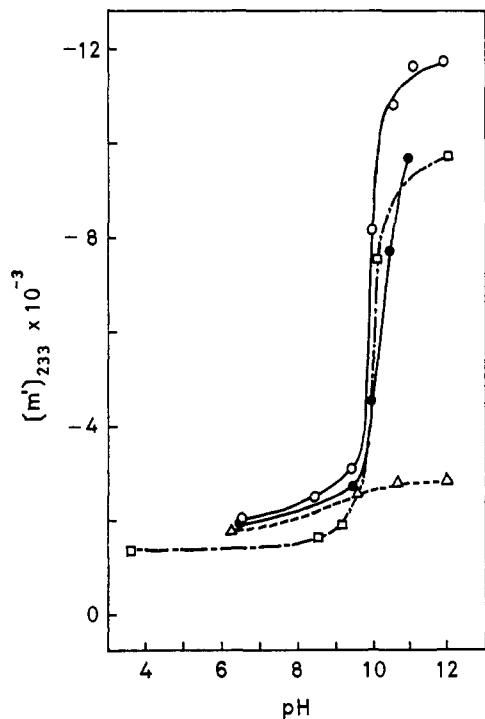

Figure 2. pH dependence of $[m']_{233}$ of poly-α-amino acids in aqueous solutions: O, poly-L-lysine (160); ●, poly-L-lysine (46); □, poly-L-ornithine (190); △, poly(L-α,γ-diaminobutyric acid) (110). Refer to Figure 1 for the meaning of the numbers in parentheses.

Poly-α-amino Acids with N-Carbobenzoxyamino Groups in the Side Chains. Figure 6 gives the results obtained from the helix–coil transitions of poly-ϵ-N-
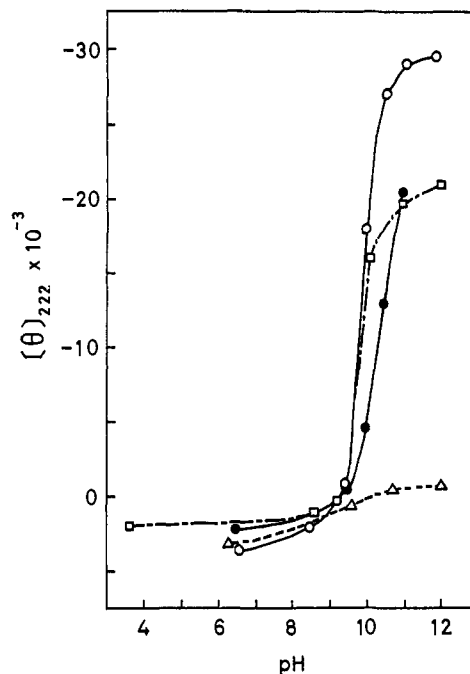

Figure 3. pH dependence of $[\theta]_{222}$ of poly-α-amino acids in aqueous solutions: O, poly-L-lysine (160); ●, poly-L-lysine (46); □, poly-L-ornithine (190); △, poly(L-α,γ-diaminobutyric acid) (110). Refer to Figure 1 for the meaning of the numbers in parentheses.

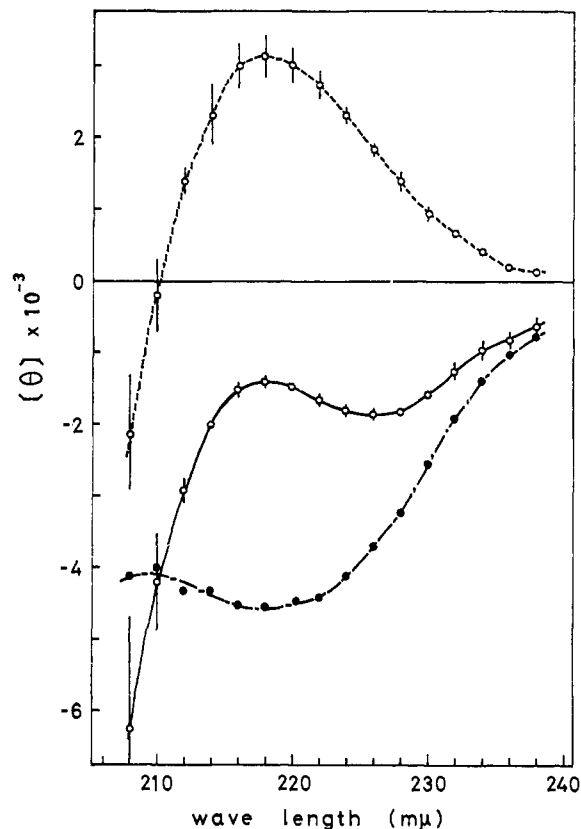Figure 4. Circular dichroism spectra of poly(L-α,γ-diaminobutyric acid) in aqueous solution at pH 7.3 (- - -O- - -) and pH 11.8 (—O—). The curve (- - -●- - -) indicates the deviation of the curve at pH 11.8 from that at pH 7.3.

carbobenzoxy-L-lysine, poly-δ-N-carbobenzoxy-L-ornithine, and poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) in a chloroform–dichloroacetic acid solvent
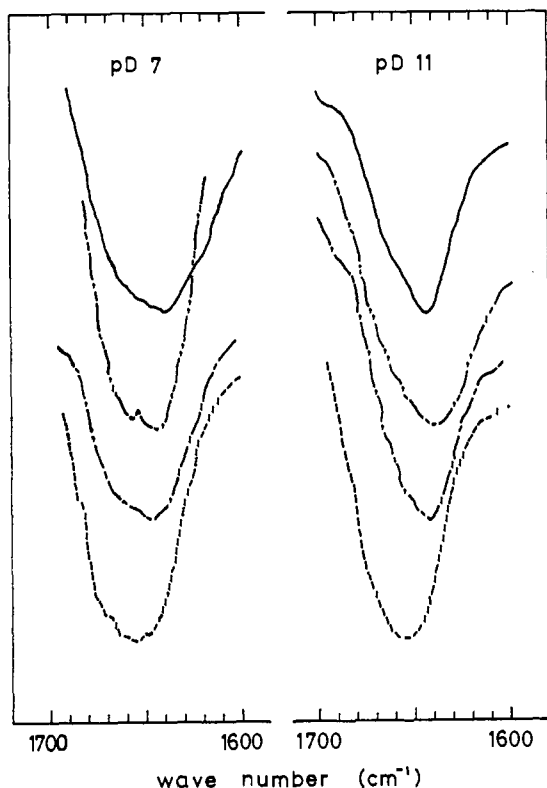
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Figure 5. Infrared spectra of poly-α-amino acids in D_2O solutions at both pD *ca.* 7 and *ca.* 11: (———), poly-L-lysine (160); (—·-·—), poly-L-lysine (46); (—·—), poly-L-ornithine (190); (-------), poly-(L-α,γ-diaminobutyric acid) (110). Refer to Figure 1 for the meaning of the numbers in parentheses.

Figure 7. Infrared spectra of poly-α-amino acids in chloroform: (———), poly-ϵ-N-carbobenzoxy-L-lysine (160); (-----), poly-δ-N-carbobenzoxy-L-ornithine (190); (------), poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) (33). The numbers in parentheses are degrees of polymerization of the polymers.
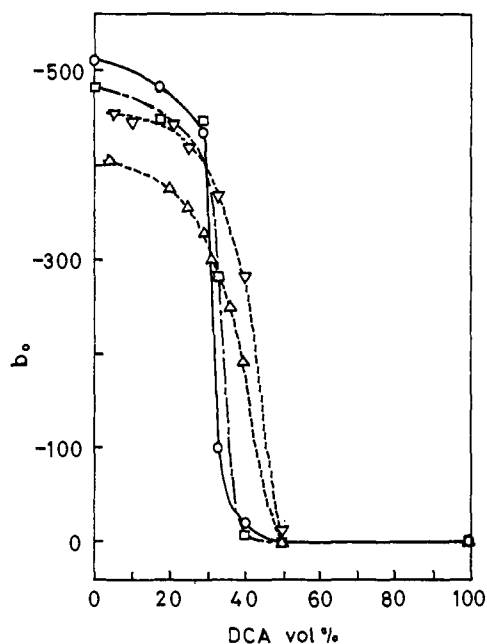

Figure 6. Solvent dependence of Moffitt's b_0 values of poly-α-amino acids in a chloroform–dichloroacetic acid solvent system: O, poly-ϵ-N-carbobenzoxy-L-lysine (300); □, poly-δ-N-carbobenzoxy-L-ornithine (190); ∇, poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) (110); △, poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) (33). The numbers in parentheses are degrees of polymerization of the polymers.

system. The previous estimation[6] that the helical structure of poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) might be the most stable of three kinds of poly-
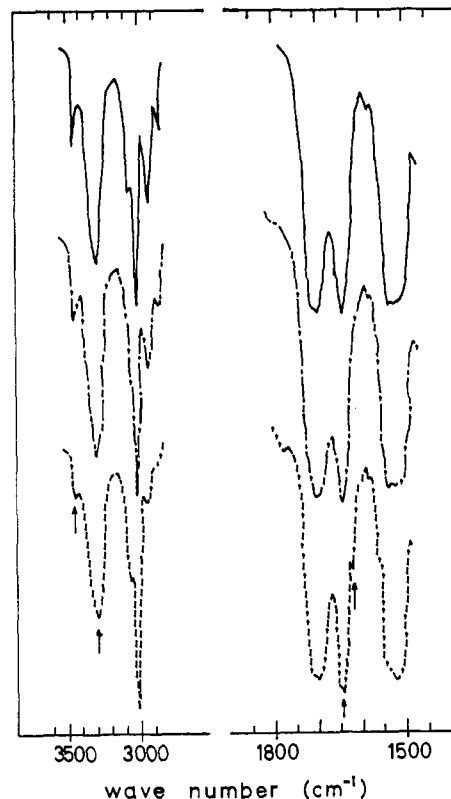
meric structure was further supported by measuring another sample of poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) with a higher degree of polymerization.

Infrared absorption spectra of the polymers in chloroform are shown in Figure 7. The amide I bands of poly-ϵ-N-carbobenzoxy-L-lysine and poly-δ-N-carbobenzoxy-L-ornithine are α bands (1655 cm^{-1}), indicating that these polymers are helical in chloroform. The amide I band of poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) also shows that in the polymer the helical structure is dominant with a small fraction of β structure (a shoulder at 1630 cm^{-1}) in chloroform,[11] where the polymer is poorly soluble. In the NH-stretching absorption region of these polymers, two bands are observed; that is, a free NH band at 3450 cm^{-1} and a hydrogen-bonded NH band at 3300 cm^{-1}. Since in the helical polymers almost all the amide NH linkages in the main chain form hydrogen bonds, the free NH bands observed in the spectra should be attributed to the urethan NH linkages in the side chains. Assuming that the absorption coefficients of all the NH-stretching vibrations in the system are equal, the molar fractions of the hydrogen-bonded NH groups of all the urethan NH groups in the side chains might be roughly estimated; for poly-ϵ-N-carbobenzoxy-L-lysine, poly-δ-N-carbobenzoxy-L-ornithine, and poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid), the fractions would be 38, 43, and 59%, respectively.

In agreement with the results obtained in the ORD and infrared absorption measurements, we propose a

(11) E. R. Blout and A. Asadourian, *J. Amer. Chem. Soc.*, 78, 955 (1956).

super-helix model, shown schematically in Figure 8, which is similar to the model proposed for poly-L-glutamic acid.[1] In the super-helix model, the hydrogen bonds between the side chains contribute to the stabilization of the helical structure. In the helical structure of poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid), which has shorter side chains, interaction between the urethan linkages in side chains through hydrogen bonds may be relatively strong.

Experimental Section

Materials. L-Glutamic acid, L-lysine, and L-ornithine were kindly supplied by Ajinomoto Co., Inc., and Kyowa Hakko Kogyo Co., Ltd., and used without further purification. Solvents were carefully purified by distillation in the usual manner.

L-α,γ-Diaminobutyric Acid Dihydrochloride. According to the method described by Adamson,[12] L-α,γ-diaminobutyric acid dipicrate, mp 179–183° dec (lit.[12] mp 178–180° dec), was obtained in 65% yield (lit.[12] 42%) from L-glutamic acid, and the dipicrate was converted into the dihydrochloride, which had mp 196–197° dec (lit.[12] 195–196° dec) and $[\alpha]^{22}D$ +14.1 (c 3.67, water) (lit.[12] $[\alpha]^{18}D$ +14.6).

Anal. Calcd for $C_4H_{12}O_2N_2Cl_2$: C, 25.14; H, 6.33; N, 14.66. Found: C, 25.49; H, 6.36; N, 14.49.

γ-N-Carbobenzoxy-L-α,γ-diaminobutyric Acid. This was prepared from L-α,γ-diaminobutyric acid dihydrochloride by the procedure of Fridecky and McGregor[13] in 48% yield (lit.[13] 36%); mp 223° dec (lit. mp 240–242° dec,[13] mp 238° dec[14]).

Anal. Calcd for $C_{12}H_{16}O_4N_2$: C, 57.13; H, 6.39; N, 11.11. Found: C, 55.98; H, 6.24; N, 10.80.

α-N-Carboxy-γ-N-carbobenzoxy-L-α,γ-diaminobutyric Acid Anhydride. This was synthesized in the usual manner[13] with phosgene and was recrystallized three times from ethyl acetate–petroleum ether; yield 64% (lit.[13] 67%); mp 60–64° (lit.[13] mp 54–59°, effervescing at 67°).

Anal. Calcd for $C_{13}H_{14}O_5N_2$: C, 56.11; H, 5.06; N, 10.07. Found: C, 56.11; H, 5.45; N, 10.00.

Poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric Acid). α-N-Carboxy-γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid anhydride was polymerized in dimethylformamide with triethylamine as an initiator, the ratio of the anhydride to the initiator being 150. Polymerization was allowed to proceed at room temperature for 5 days, whereupon water was added. The precipitate was filtered, washed with water, and dried *in vacuo;* yield 87% (lit.[13] 84%). The degree of polymerization estimated from viscometry was 110.

Anal. Calcd for $C_{12}H_{14}O_3N_2$: C, 61.52; H, 6.02; N, 11.96. Found: C, 61.37; H, 5.85; N, 11.81.

Poly(L-α,γ-diaminobutyric Acid). Poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid) was suspended in a mixture of dioxane and chloroform. Anhydrous hydrogen bromide was then passed through the solution at room temperature for 2 hr. The precipitate was filtered under nitrogen atmosphere, dried *in vacuo,* dialyzed against frequent changes of distilled water for 5 days, concentrated under reduced pressure, and dried in a frozen state *in vacuo;* yield 75%.

Poly-L-lysine and Poly-L-ornithine. ε-N-Carbobenzoxy-L-lysine and σ-N-carbobenzoxy-L-ornithine were obtained by the reaction of L-lysine and L-ornithine, respectively, with an equimolar amount of carbobenzoxy chloride in the presence of sodium hydroxide. These ω-N-carbobenzoxylated amino acids were converted into the corresponding polymers by the same method as described for the synthesis of poly(L-α,γ-diaminobutyric acid).

Estimation of Degrees of Polymerization (DP's). For the estimation of DP's of the N-carbobenzoxylated polymers, the equation[15] $\log DP = 1.47 \log [\eta]^{25}DCA + 2.99$ was used, where $[\eta]^{25}DCA$ is an intrinsic viscosity measured in dichloroacetic acid at 25.0°.

For the sample of poly-L-lysine derived from poly-ε-N-carbobenzoxy-L-lysine (with the DP of 46) by decarbobenzoxylation and
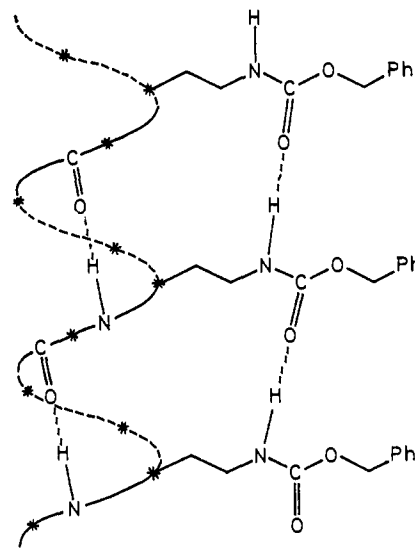
(12) D. W. Adamson, *J. Chem. Soc.,* 1564 (1939).
(13) M. J. Fridecky and W. H. McGregor, *J. Med. Chem.,* 9, 255 (1966).
(14) T. Kurihara and K. Suzuki, *J. Pharm. Soc. Jap.,* 75, 1269 (1955).
(15) M. Hatano, *et al.,* unpublished data.

Figure 8. Scheme of a possible partial super-helix structure of poly(γ-N-carbobenzoxy-L-α,γ-diaminobutyric acid).

dialysis, the DP was estimated to be 79 from the viscosity measured in water at 25.0° by the aid of the equation[16] $\log DP = 0.79 \log \eta_{sp} + 2.46$, where η_{sp} is a specific viscosity at the concentration of the polymer of 1%. For the other decarbobenzoxylated polymers the viscosities were not measured; however, the DP's of these polymers would be higher than those of the corresponding N-carbobenzoxylated polymers since a portion of the polymer with lower DP was lost during dialysis.

Optical Rotatory Dispersion and Circular Dichroism Measurements. The ORD and CD measurements of the decarbobenzoxylated polymers were carried out at 20° with a JASCO Model ORD/UV-5 optical rotatory dispersion recorder with a CD attachment, using cells of path lengths of 0.1, 1, and 10 mm. The concentration range of the polymers was 0.1–0.2 g/dl. The Moffitt's b_0 values were determined from the data obtained over the wavelength range of 250–500 mμ, assuming a λ_0 of 212 mμ. The dependency of the refractive index of water upon wavelength was taken into consideration. Reduced mean residue rotation at 233 mμ, $[m']_{233}$, in deg cm²/dmol and residue ellipticity at 222 mμ, $[\theta]_{222}$, in deg cm²/dmol were also calculated.

The ORD measurements of the N-carbobenzoxylated polymers were carried out over the wavelength range of 290–500 mμ at 25° using a 10-mm cell. The concentration range of the polymers was 0.2–1.0 g/dl. The curves obtained were analyzed with the Moffitt equation, assuming a λ_0 of 212 mμ and refractive indices of the mixed solvents to be average values of those of the two pure solvents at 589 mμ allotted in proportion to their volume fractions.

Infrared Spectral Measurement. The infrared spectra of the decarbobenzoxylated polymers in D_2O solutions were recorded on Perkin-Elmer Model 125 grating infrared spectrophotometer with small bags made of polyethylene film held between NaCl plates under a nitrogen atmosphere. The solvent was prepared by dissolving sodium hydroxide in D_2O and the pH values of the solutions were determined with Toyo pH test paper from Toyo Roshi Kaisha, Ltd.

The infrared spectra of the solutions of the N-carbobenzoxylated polymers in chloroform were recorded on Hitachi Model EPI-G2 grating infrared spectrophotometer with NaCl cells of path length of 0.1 mm.

(16) A. Yaron and A. Berger, *Biochim. Biophys. Acta,* 69, 397 (1963).