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NOTE

^{13}C NMR Studies of Solvent-induced Helix-Coil Transitions of Poly(γ-phenethyl-L-glutamate) and Poly[γ-(3-phenylpropyl)-L-glutamate]

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Although much simpler in structure than proteins, synthetic polypeptides have served as models for studying various conformational features of proteins. High resolution nuclear magnetic resonance (NMR) spectroscopy has been extensively applied to the study of conformations and conformational transitions of oligo- and polypeptides, particularly their helix-coil transitions. A wide range of carbon-13 chemical shift is especially useful for obtaining information on carbon atoms in amino acid residues such as those in the backbone and side chains. In NMR studies on conformational changes in proteins and polypeptides, the magnitude and sign of the chemical shift displacements induced by external perturbations provide important information. Thus it is important to find the general rule underlying chemical shift displacements accompanying the helix-coil transition of polypeptides.

In preceding papers, the helix-coil transitions of poly(γ-benzyl L-glutamate) [poly(Glu(OBzl))],[1] poly(β-benzyl L-aspartate) [poly(Asp(OBzl))],[2] and poly(γ-methyl L-glutamate) [poly(Glu(OMe))][3] in trifluoroacetic acid (F_3AcOH)/deuterated chloroform ($CDCl_3$) solvent mixtures were studied by NMR, and some regularity was found to exist in the order of chemical shift displacements along the side chains of these polypeptides. The solvent-induced shifts of carbons in the model compounds were compared with those in polypeptides, and it was concluded that the conformational changes play a dominant role in the chemical shift displacements of amido, α, β, and γ carbon atoms in polypeptides, while the chemical shift displacements of the remaining side chain carbon atoms may be attributed only to the interaction between the side chain ester group and F_3AcOH.[3-5]

This paper reports the findings of a ^{13}C NMR study on the helix-coil transitions of poly(γ-phenethyl L-glutamate) [poly(Glu(OPE))] and poly[γ-(3-phenylpropyl)-L-glutamate] [poly(Glu(OPP))] carried out to check the generality of the above conclusion. These polypeptides differ from poly[Glu(OBzl)] only with respect to the length between the ester and phenyl groups of the side chains. The effect of the side chain length on the helix stability is also discussed on the basis of data obtained for a series of poly(L-glutamate)s.

EXPERIMENTAL

Samples of poly(Glu(OPE)) and poly(Glu(OPP)) were prepared by polymerizing *N*-carboxyanhydrides (NCA) of Glu(OPE) and Glu(OPP) in dioxane at 25°C using triethylamine as an initiator. The α-CH proton resonances of these polypeptides in $F_3AcOH/CDCl_3$ at 100 MHz in the region of helix-coil transition appeared essentially as single shifting peaks and no "double-peak" phenomenon was observed. These results indicated that the average molecular weights of our polypeptide samples were sufficiently high. The poly(Glu(OPE)) sample was supplied by the courtesy of Mr. M. Nagao and Professor I. Uematsu of the Tokyo Institute of

Technology, and the sample of poly(Glu(OBzl)) used was the same as that described elsewhere[1,4] and had an average degree of polymerization of about 820. Carbon-13 NMR spectra were obtained on a JEOL JNM PS-100 spectrometer equipped with a PFT-100 Fourier transform system and a noise-modulated proton decoupler operating at 25.1 MHz and 35°C. Chemical shifts were measured as downfield shifts from the signal for tetramethylsilane (TMS) as an internal reference. Optical ro-

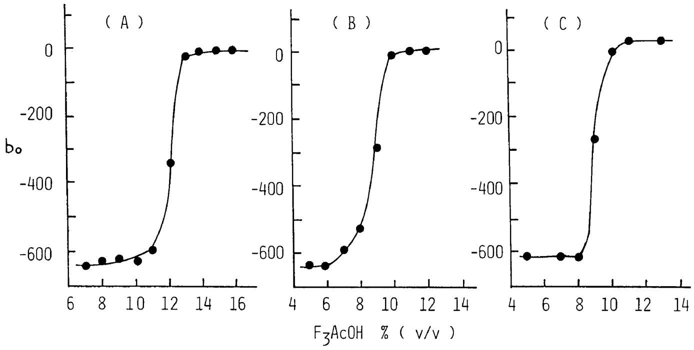

Figure 1. Dependence of Moffitt–Yang parameter b_0 on solvent composition. (A) poly(Glu(OBzl)), (B) poly(Glu(OPE)), and (C) poly(Glu(OPP)) in $F_3AcOH/CDCl_3$ mixtures containing 5% (w/v) polymer at 25°C.

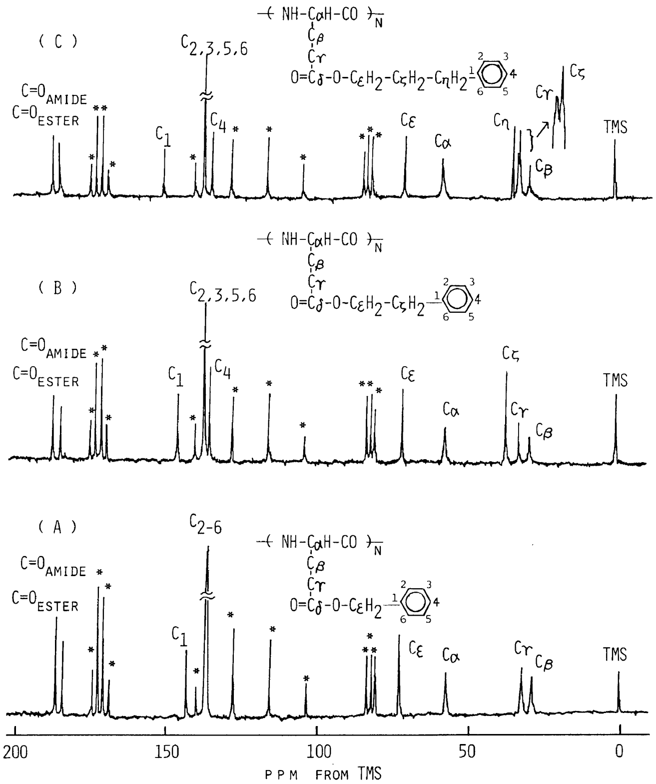

Figure 2. ^{13}C-NMR spectra of (A) poly(Glu(OBzl)), (B) poly(Glu(OPE)), and (C) poly(Glu(OPP)) with coiled conformations in 15% (v/v) F_3AcOH/85% (v/v) $CDCl_3$ mixture containing 5% (w/v) polymer at 35°C and 25 MHz. The resonances assigned to solvents are marked by an asterisk.

tatory dispersion (ORD) measurements were performed at 25°C on a Jasco J-20 spectrometer equipped with a cylindrical cell of 1 mm light pass length. The polymer concentration for the ^{13}C NMR and ORD measurements was 5% (w/v).

RESULTS AND DISCUSSION

The Moffitt-Yang parameter b_0 provides information on the conformation of a polypeptide in solution.[6] For polymers of L-amino acid, the b_0 value for a perfect α-helix was taken to be −630 and that for random coil, zero.[7] The dependence of b_0 on the solvent composition is shown in Figure 1 for poly(Glu(OBzl)), poly(Glu(OPE)), and poly(Glu(OPP)) in $F_3AcOH/CDCl_3$ mixtures. The drastic change in b_0 indicates the occurrence of a sharp helix-coil transition. The solvent dependence of b_0 indicates that all the polypeptides investigated here take on a right-handed perfect α-helical conformation and a coiled conformation at lower and higher F_3AcOH concentrations, respectively. The F_3AcOH concentration at the midpoint of the helix-coil transition was 12% (v/v) for poly(Glu(OBzl)) and 9% (v/v) for poly(Glu(OPE)) and poly(Glu(OPP)). In Figure 2 are shown the ^{13}C NMR spectra of poly(Glu(OBzl)), poly(Glu(OPE)), and poly(Glu(OPP)) having coiled conformations in

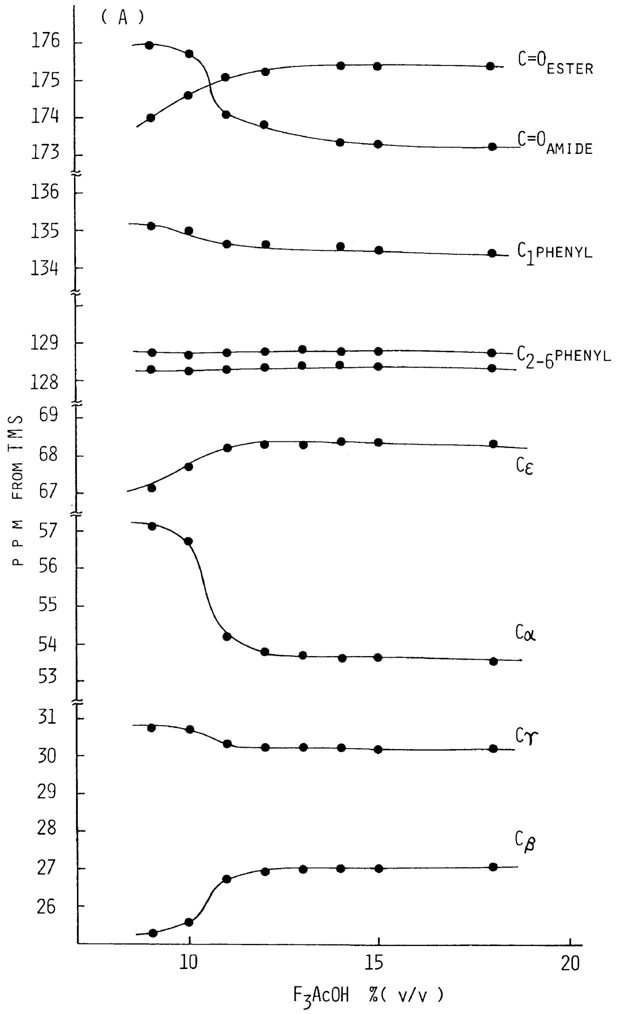

Figure 3

a 15% F_3AcOH/85% $CDCl_3$ (v/v) mixture at 35°C. The structures of the monomers and the notation of their carbon atoms are also shown in Figure 2. All the peaks in the ^{13}C spectra of poly(Glu(OPE)) and poly(Glu(OPP)) were assigned on the basis of a comparison with the peak assignments of poly(Glu(OBzl)). The distinction between the C_γ and C_ζ resonances of poly(Glu(OPP)) was made on the assumption that as the side chain carbons become more and more separated from the backbone, they take on greater mobility, thus giving a narrower resonance line. This assumption is based on the NMR spectrum of poly(Glu(OPE)). It should be noted that the phenyl C_4 resonances of poly(Glu(OPE)) and poly(Glu(OPP)) are well resolved, while that of poly(Glu(OBzl)) is superimposed on the $C_{2,3,5,6}$ resonances.

In Figure 3, the ^{13}C chemical shifts are plotted against the concentration of F_3AcOH in F_3AcOH/$CDCl_3$ mixtures. The helix-coil transition appears in each plot as an inflection. The backbone carbons C_α and $C=O_{\mathrm{amido}}$ and the side chain carbon C_β show large down- and up-field shifts, respectively, in the region of coil-to-helix transition. Most of the other side chain carbons also show significant shifts in the transition region. The F_3AcOH concentration at the midpoint of the helix-coil transition determined from the chemical shift displacements was

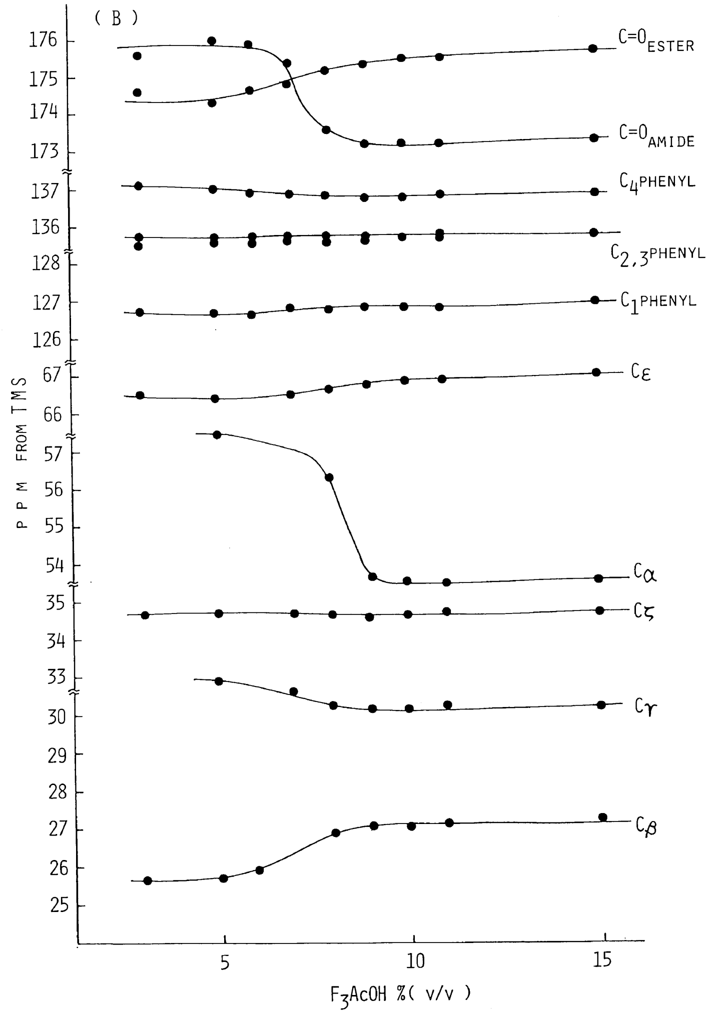

Figure 3

about 10.5% (v/v) for poly(Glu(OBzl)) and 7.5% (v/v) for poly(Glu(OPE)) and poly(Glu(OPP)). The sharpness of the chemical shift displacement for each carbon in the transition region was different, but the midpoint F_3AcOH concentrations for both backbone and side chain carbons of a given polypeptides agreed well with each other. However, the midpoint F_3AcOH concentrations were about 1.5% (v/v) lower than those determined from the changes in the ORD parameter b_0. The only difference in the conditions between NMR and ORD measurements was the temperature, *i.e.*, 35°C for the former and 25°C for the latter. Since poly(Glu(OBzl)) has a higher helical stability at higher temperature,[1,10] the midpoint F_3AcOH concentration determined from NMR must be higher than that from ORD. The discrepancy between the results from NMR and ORD cannot be explained at present.

The midpoint solvent composition is not too sensitive to the molecular weight of polypeptide when the molecular weight is sufficiently high.[8] Hence, the dependence of the stability of helical conformation on the side chain length can be judged in terms of the midpoint solvent composition.

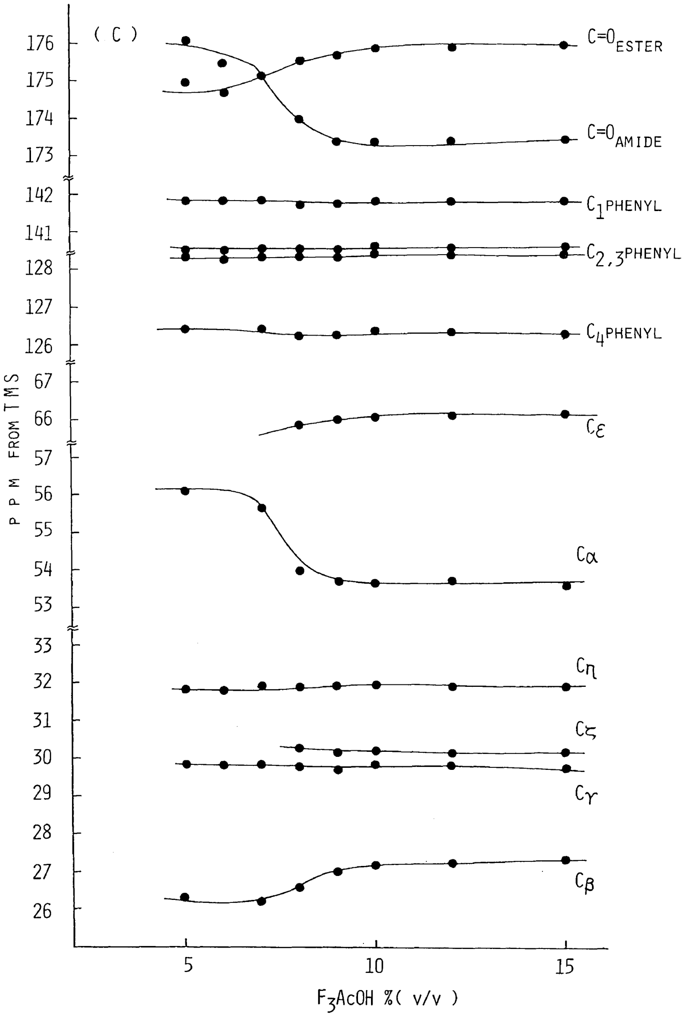

Figure 3. ^{13}C chemical shift displacements of all carbon atoms of (A) poly(Glu(OBzl)), (B) poly(Glu(OPE)), and (C) poly(Glu(OPP)) as a function of the solvent composition in $F_3AcOH/CDCl_3$ mixture containing 5% (w/v) polymer at 35°C.

Table I. ^{13}C Chemical shift differences $\Delta\delta$ (ppm)[a] in $F_3AcOH/CDCl_3$ mixtures

Carbon	Poly(Glu(OBzl))	Poly(Glu(OBzl))	Poly(Glu(OPE))	Poly(Glu(OPP))
	15 w/v%	5 w/v%	5 w/v%	5 w/v%
$C=O_{amido}$	−2.7	−2.7	−2.8	−2.6
C_α	−3.3	−3.4	−3.8	−2.4
C_β	2.0	1.8	1.4	1.1
C_γ	−0.5	−0.6	−0.8	—[b]
$C=O_{ester}$	1.7	1.5	1.1	1.4
O	?	?	?	?
C_ε	1.4	1.3	0.5	0.6
C_ζ	—	—	−0.1	−0.1
C_η	—	—	—	0.1
$C_{1\ phenyl}$	−0.4	−0.4	0.2	0
$C_{2,3,5,6\ phenyl}$	0.1	0.2	0.0_5	0.1
$C_{4\ phenyl}$	0.1	0.2	0.1	0.1

[a] $\Delta\delta=\delta_{coil}-\delta_{helix}$.

[b] Not determined accurately due to C_ζ resonance overlapping.

According to this criterion, poly(Glu(OBzl)) is the most stable among the three poly-L-glutamates investigated here and poly(Glu(OPE)) and poly(Glu(OPP)) are equally stable against the helix-breaking solvent F_3AcOH. It has been suggested[9] that since the bulky phenyl group shields the intramolecular hydrogen-bonded backbone more efficiently from F_3AcOH than alkyl group, the poly(Glu(OBzl)) helix is more stable than the poly(Glu(OMe)) helix. The shielding power of the phenyl group may be reduced in poly(Glu(OPE)) and poly(Glu(OPP)). This interpretation is only approximate, since the helical stability may be subtly dependent on a variety of factors, such as interactions between side chains, solvation of ester group, and thermal fluctuations of the side chain.

In Table I are summarized the chemical shift differences $\Delta\delta=\delta_{coil}-\delta_{helix}$, where δ_{coil} and δ_{helix} are the chemical shifts in the coil and helical conformations, respectively, and the minus sign indicates the downfield shift in the coil-to-helix transition. The $\Delta\delta$ values for poly(Glu(OBzl)) at a polypeptide concentration of 15% (w/v) are also shown in Table I. No significant concentration dependences of $\Delta\delta$ can be seen for this polypeptide. Table I shows that the sign and magnitude of $\Delta\delta$ for non-aromatic carbons in poly(Glu(OPE)) and poly(Glu(OPP)) obey the same rule as found previously for poly(Glu(OBzl)).[3-5] Thus large negative values of $\Delta\delta$ are obtained for the backbone C_α and $C=O_{amido}$ and $\Delta\delta$ alternates its sign along the side chain when the hypothetical chemical shift difference of the oxygen atom is negative. Carbons having the same subscript have the same sign. This regularity of the chemical shift displacements in the three poly(L-glutamate)s studied indicates that the chemical shift displacements accompanying the solvent-induced helix-coil transition have the same origin as that proposed previously for poly(Glu(OBzl)).[3-5] Thus, for these three polypeptides, it may be concluded that the conformational changes are the major origin of the chemical shift displacements of amido, C_α, C_β, and C_γ carbon atoms, while the interaction between F_3AcOH and the side chain ester group is primarily responsible for the chemical shift displacements of other non-aromatic side chain carbon atoms. The sign and magnitude of $\Delta\delta$ of the carbon atoms constituting the ester portion may be roughly explained by the σ-inductive effect.[11]

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