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Conformational Characterization of Solid Polypeptides by ^{13}C NMR Recorded by the Cross Polarization–Magic Angle Spinning Method: Conformation-Dependent ^{13}C Chemical Shifts of Oligo- and Poly(γ-benzyl L-glutamates) and Sequential Copolymers of γ-Benzyl and γ-Methyl L-Glutamates and Qualitative Evaluation of Side-Chain Orientation

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ABSTRACT: We have recorded ^{13}C NMR spectra of oligo(γ-benzyl L-glutamates), poly(γ-benzyl L-glutamates) ([Glu(OBzl)]$_n$), and copolymers of γ-benzyl and γ-methyl L-glutamates in the solid state by the cross polarization–magic angle spinning method, in order to elucidate conformational features as viewed from the conformation-dependent ^{13}C chemical shifts. It is found that the relative ^{13}C chemical shifts of the α-helix with respect to those of the β-sheet form are 5.2, −3.4, and 4.6 ppm for the C_α, C_β, and amide carbonyl carbons, respectively, for [Glu(OBzl)]$_n$. For the sequential copolymers consisting of γ-benzyl L-glutamate (B) and γ-methyl L-glutamate (M), we found that the α-helix conformation is achieved when the proportion of B is over 50% regardless of their sequences. On the contrary, the β-sheet form is obtained when M is dominant. There appears no significant displacement of the ^{13}C chemical shifts for the sequential copolymers from those of [Glu(OBzl)]$_n$. Observation of differential line broadening among carbons in the backbone and side-chain moiety was found to be an excellent means to examine the orientation of the side chains. In particular, we found that considerably disordered side chains, as viewed from the selective line broadening, are characteristic of the β-sheet conformation for both the homopolypeptides and the sequential copolymers.

Introduction

It is now obvious from our previous studies[1-4] as well as others[5-10] that ^{13}C chemical shifts of solid polypeptides, as determined by the cross polarization–magic angle spinning (CP–MAS) technique, exhibit substantial extents of conformation-dependent ^{13}C chemical shifts (up to 7 ppm) depending on particular conformations such as α-helix and β-sheet forms. It appears that such conformation-dependent ^{13}C shifts mainly arise from the local conformation of individual amino acid residues characterized by the dihedral angles (Φ and Ψ) as well as the manner of intra- or intermolecular hydrogen bonding, as suggested by the previous theoretical calculation of the ^{13}C chemical shifts utilizing the FPT–INDO molecular orbital method.[11] Tonelli previously noted that the ^{13}C NMR chemical shifts of polypeptides should be and are sensitive to their local conformations.[12] Peptide residue sequence effects on the ^{13}C NMR chemical shifts, when they occur, are caused by the dependence of the local conformation on the sequence, as, for example, in those residues that precede proline in the polypeptide sequence. Such dependence of the ^{13}C chemical shifts upon the local conformation is one of the major advantages of ^{13}C NMR as a tool for conformational characterization of complicated peptides and proteins,[3,7,13,14] although prior knowledge with regard to how and to what extent the ^{13}C chemical shifts are displaced by particular conformations is required. For this purpose, it is essential to utilize the ^{13}C chemical shifts of a variety of polypeptides or peptides with known dihedral angles as determined by X-ray diffraction for a source of reference data.

As a continuation of our effort to relate the conformation-dependent ^{13}C chemical shifts of polypeptides to their particular conformation in the solid state, we aimed, in this paper, to analyze ^{13}C NMR spectra of monodisperse molecular weight oligo(γ-benzyl L-glutamates), poly(γ-benzyl L-glutamates) ([Glu(OBzl)]$_n$ or (B)$_n$), and sequential copolymers of γ-benzyl and γ-methyl L-glutamates. α-Helical

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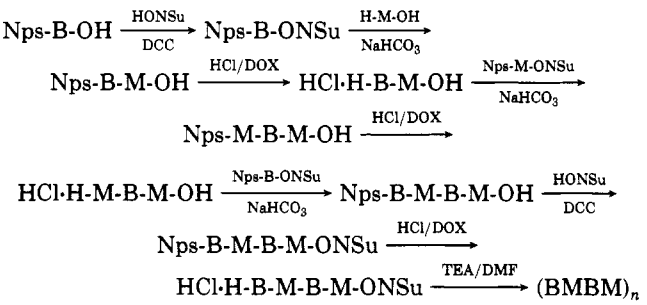
$[Glu(OBzl)]_n$ is recognized as a cylindrical rod supported by an inner core with considerable rigidity sheathed in a soft outer core of flexible side chains.[15] As an aid in understanding the role of the side-chain moiety in stabilizing such a helix, it is of particular interest to analyze the sequential copolymers. For this purpose, it is emphasized that the ^{13}C CP-MAS NMR approach as a tool for conformational characterization is useful not only for crystalline materials but also for samples whose crystalline packing is very poor.

Experimental Section

Materials. A series of monodisperse oligo(γ-benzyl L-glutamates), Nps-[L-Glu(OBzl)]$_n$-NHCH$_2$CH$_2$CH$_2$CH$_3$ (n = 1–10 and 16) containing an n-butylamide group at the C-terminal residue and an (o-nitrophenyl)sulfenyl (Nps) group at the N-terminal residue, were synthesized according to the procedure described in the previous papers.[16,17] They were isolated and purified by recrystallization.

Sequential polypeptides consisting of γ-benzyl L-glutamate (B) and γ-methyl L-glutamate (M), (MBBB)$_n$, (BBM)$_n$, (MMBB)$_n$, (BMBM)$_n$, (MMB)$_n$, and (BMMM)$_n$, were prepared by the dicyclohexylcarbodiimide (DCC)–N-hydroxysuccinimide (HONSu) coupling method.[16,18-20] The synthetic route of (BMBM)$_n$, for example, is shown in the following scheme.



In the scheme, DOX, TEA, and DMF stand for 1,4-dioxane, triethylamine, and dimethylformamide, respectively. After being treated by the conventional procedure, the product (BMBM)$_n$ was fractionated by gel filtration on Sephadex G-50 (column, 2.5 × 90 cm; flow rate, 0.9 mL/min; solvent, dimethyl sulfoxide), and the α(BMBM)$_n$ (fractions of higher molecular weight) and the β(BMBM)$_n$ (lower molecular weight) were collected.

Poly(γ-benzyl L-glutamates) and poly(γ-methyl L-glutamate) were prepared by the polymerization of γ-benzyl and γ-methyl L-glutamate N-carboxyanhydride (NCAs) in dichloroethane using triethylamine as initiator, respectively.[21-23] The products were recrystallized from dichloroethane/methanol and then extracted with methanol by using a Soxhlet extractor to remove low molecular weight polymers. Characteristics of the samples used here are summarized in Table I.

Method. Single-contact ^{13}C CP-MAS NMR spectra[25,26] were recorded by a Bruker CXP-300 spectrometer at 75.46 MHz equipped with a CP-MAS accessory. Samples were placed in an Andrew-Beams-type rotor machined from perdeuterated poly(methyl methacrylate) and spun as fast as 3–4 kHz. A contact time of 800 μs was chosen to avoid a buildup of residual signals from the rotor and probe assembly. Those residual peaks were subtracted digitally from phase-corrected spectra. Repetition time was 2 s, and spectral width and data points were 30 kHz and 4K points, respectively. Spectra were accumulated more than 2000 times. Chemical shifts were calibrated through external benzene and converted to the value from tetramethylsilane (Me_4Si).

Infrared spectra were recorded with Hitachi Model 260-50 and Jasco A-702 infrared spectrophotometers for KBr disks or films cast from dichloroacetic acid/diethyl ether.

Results and Discussion

Oligo(γ-benzyl L-glutamates) and Poly(γ-benzyl L-glutamates). Figure 1 illustrates the ^{13}C CP-MAS NMR spectra of a series of monodisperse oligo(γ-benzyl L-glutamates), Nps-[L-Glu(OBzl)]$_n$-NHBu (n = 1–10, 16), and poly(γ-benzyl L-glutamates), H-[L-Glu(OBzl)]$_n$-OH, in the solid state. Assignment of peaks for the Glu(OBzl) residue is straightforward in view of data taken in CF_3COOH solution[27,28] and is in good agreement with recent data for the solid state obtained by Kricheldorf et al.[6,9] The assignment of the n-butylamide peak is easily performed by taking into account that the peak intensities gradually decrease with increasing DP_n (degree of polymerization) for Glu(OBzl) residues in the oligomers. On the other hand, it is worth noting that the C_α peak of the N-terminal Glu(OBzl) residue of the very lowest oligomers appears in a region between 55 and 60 ppm, which is different from that of the other residues (ca. 50 ppm).

Recently, Kricheldorf et al. attempted to relate the conformational behavior of the $[Glu(OBzl)]_n$ with average degree of polymerization (DP_n).[6,9] Such an attempt, however, may not succeed because of the polydispersity of the samples employed. In this study, we analyzed a series of monodisperse homooligomers to clarify the relationship mentioned above. From the plot of the ^{13}C chemical shifts vs. DP_n given in Figure 2, it is obvious that the ^{13}C chemical shifts of the C_α and carbonyl (amide) carbons are displaced downfield at DP_n around 11–16 by 5.2 and 4.6 ppm, respectively, while the C_β ^{13}C shift is displaced upfield by 3.4 ppm. Such behavior is ascribed to the conformational change from the β-sheet form to the α-helix form. This trend is also consistent with the observed change of infrared spectra with increasing number of Glu(OBzl) residues, as shown in Figure 3. It is seen from Figure 3 that the oligomers with DP_n between 4 and 10 take the β-sheet form manifested by characteristic peaks of 1690, 1630, and 1530 cm^{-1}, and the oligomer with DP_n = 16 and polymers adopt the α-helix conformation as characterized by observation of bands at 1656 and 1545 cm^{-1}. It should be pointed out that the amide C=O chemical shift of the β-sheet form is superimposed on the signal of the ester C=O group.

For the very lowest oligomers with $DP_n \leq 3$, the ^{13}C chemical shifts of the C-terminal (n-butylamide) group and the N-terminal ((o-nitrophenyl)sulfenyl; around 130 ppm) group vary gradually with the number of residues and reach constant values in the tetramer. As shown in Figure 4, the ^{13}C chemical shifts of the n-butylamide group are displaced irregularly in going from the monomer to the trimer and assume constant values at the tetramer. This behavior is quite different from that observed in a series of oligo(L-alanines),[2] probably due to the difference in

Table I
Characteristics of the Samples Used

sample	DP_n ($\overline{DP}_n$)	M comp, %	$[\eta]$,[b] dL/g
Nps-[L-Glu(OBzl)]$_n$-NH(CH$_2$)$_3$CH$_3$	1–10, 16		
H-[L-Glu(OBzl)]$_n$-OH			
PBLG-1	(<200)		
PBLG-2	(250)[c]		
H-[L-Glu(OMe)]$_n$-OH			
sequential polypeptides[a]			
(MBBB)$_n$		25	0.14
(BBM)$_n$		33	0.11
(MMBB)$_n$		50	0.17
(BMBM)$_n$		50	0.12
(MMB)$_n$		67	0.10
(BMMM)$_n$		75	0.14

[a] Abbreviations: B, γ-benzyl L-glutamate; M, γ-methyl L-glutamate. [b] Intrinsic viscosity $[\eta]$ of the sequential copolymers was measured by an Ubbelohde-type viscometer in dichloroacetic acid at 25 °C. [c] $\overline{DP}_n$ was estimated from the following equations: $[\eta] = 2.78 \times 10^{-5}\bar{M}_w^{0.87}$ (Doty et al.[24]) and $\bar{M}_w = 219 \times \overline{DP}_n$.

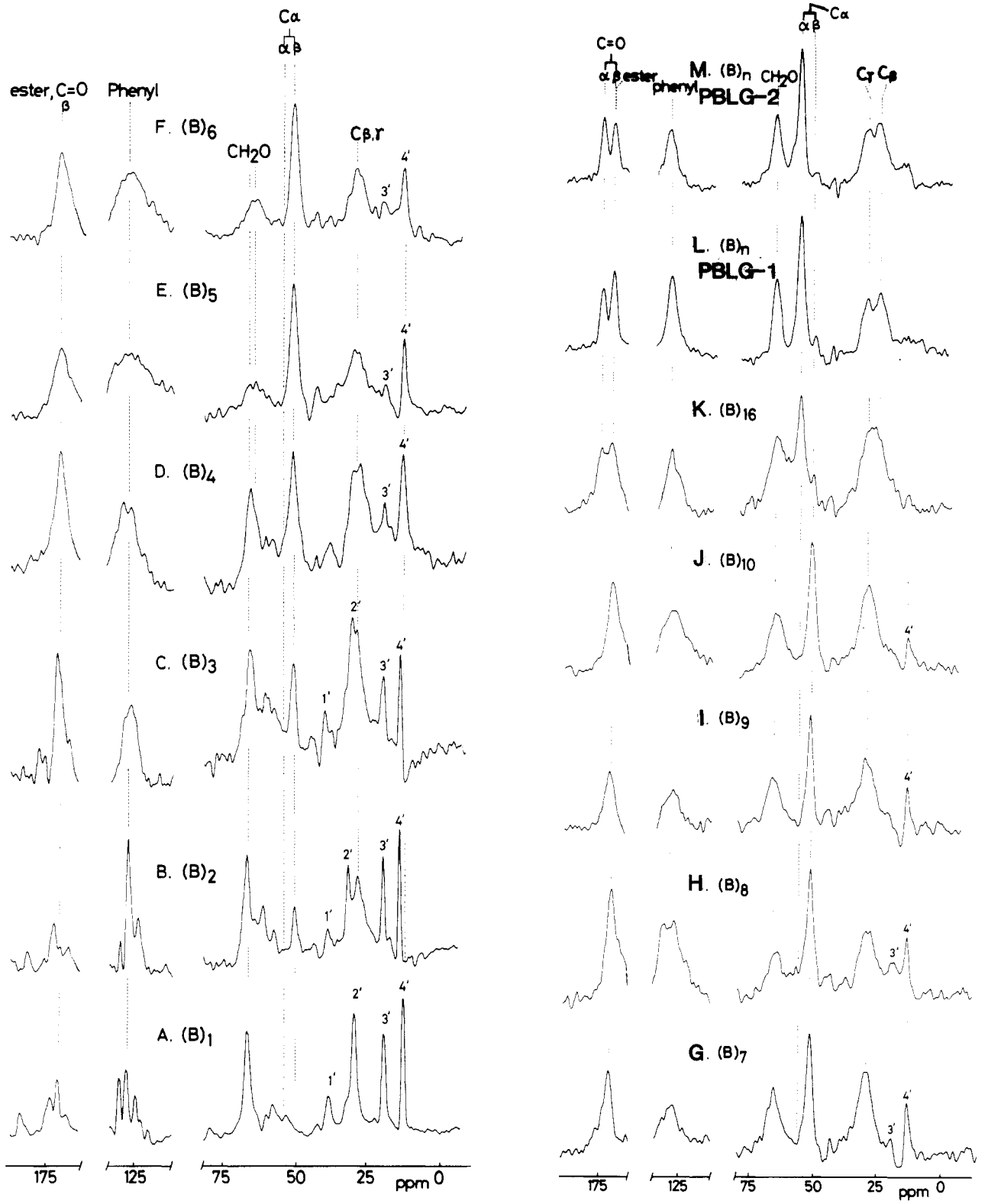


Figure 1. 75.46-MHz ^{13}C CP–MAS NMR spectra of solid oligo(γ-benzyl L-glutamates) (A–K) and of poly(γ-benzyl L-glutamates) (L, M). Peaks indicated by the primed numbers are from the *n*-butylamide group at the C-terminal group.

"size" of the side chains. Nevertheless, the displacement of the ^{13}C chemical shifts of the *n*-butylamide group is an excellent indicator that conformations which are different from the α-helix and the β-sheet forms are present in the oligomers with $DP_n \leq 3$. This finding is in agreement with that observed by infrared spectra.

Sequential Copolymers of γ-Benzyl and γ-Methyl L-Glutamates. As shown in Figure 5, ^{13}C CP–MAS NMR spectra of the sequential copolymers consisting of γ-benzyl (B) and γ-methyl L-glutamates (M) give rise to spectral features very similar to those found in the homopolypeptides as described above and also by Kricheldorf.[9] In particular, the ^{13}C chemical shifts of individual signals, as shown in Figure 6, are in good agreement with those of $(B)_n$ summarized in Table II, although the methyl signal from the M residue is superimposed on the C_α signal of the β-sheet conformation.[6] This result implies that no particular conformations other than the α-helix and β-sheet forms are present in the copolymers, nor are there any effects of sequence on the ^{13}C chemical shifts.

Table II
^{13}C Chemical Shifts Characteristic of the α-Helix and β-Sheet Forms (±0.5 ppm from Me_4Si)

	C_α			C_β			C=O			
polypeptide	α-helix	β-sheet	Δ^a	α-helix	β-sheet	Δ^a	α-helix	β-sheet	Δ^a	ref
$(Ala)_n$	52.4	48.2	4.2	14.9	19.9	-5.0	176.4	171.8	4.6	2
	52.3	48.7	3.6	14.8	20.0	-5.2	176.2	171.6	4.6	8
	52.8	49.3	3.5	15.5	20.3	-4.8	176.8	172.2	4.6	9
$(Leu)_n$	55.7	50.5	5.2	39.5	43.3	-3.8	175.7	170.5	5.2	1, 2
	55.8	51.2	4.6	43.7[d]	39.6[d]	(4.1)	175.8	171.3	4.5	9
$[Glu(OBzl)]_n$	56.4	51.2	5.2	25.6	29.0	-3.4	175.6	171.0	4.6	this work
	56.8	51.1	5.7	25.9	29.7	-3.8	175.4	172.2	3.2	9
$[Glu(OMe)]_n$	57.0	51.4	5.6				175.7	172.5	3.2	this work
	47.0[d]	51.2	(-4.2)	26.3	29.8	-3.5	175.9	172.2	3.7	9
$(B, M)_n$[b]	56.4	50.8	5.6	25.7	28.5	-2.8	176.1	171.7	4.4	this work
$[Asp(OBzl)]_n$	53.4	49.2	4.2	33.8	38.1	-4.3	174.9	169.8	5.1	4
	53.6			34.2			174.9			9
$(Val)_n$	65.5	58.4	7.1	28.7	32.4	-3.7	174.9	171.8	3.1	1
		58.2			32.4			171.5		9
$(Ile)_n$	63.9	57.8	6.1	34.8	39.4	-4.6	174.9	172.7	2.2	1
		57.1			33.1			171.0		9
$(Lys)_n$[c]	57.4			29.9			176.5			43
$[Lys(Z)]_n$	57.6	51.4	6.2	29.3	28.5	-0.8	175.7	170.4	5.3	9
$(Arg)_n$[c]	57.1			28.9			176.8			43
$(Phe)_n$	61.3	53.2	8.1	35.0	39.3	-4.3	175.2	169.0	6.2	9
$(Met)_n$	57.2	52.2	5.0	30.2	34.8	-4.6	175.1	170.6	4.5	9
$(Tyr)_n$	54.8	52.1	6.5	36.1	39.3	-3.2	176.7	169.6	7.1	9
	~58.6		~2.7							
$(Gly)_n$		43.2						168.4		3
		44.3						169.2		9
			5.1 ± 1.0[e]			-4.1 ± 0.7[e]	175.8 ± 0.8[e]	170.9 ± 1.2[e]	4.5 ± 1.1[e]	

[a] Difference of the ^{13}C chemical shifts of the α-helix relative to those of the β-sheet form. [b] Average of several sequential copolymers consisting of Glu(OBzl) and Glu(OMe). [c] Data taken in neutral aqueous solution. [d] Mistyping or erroneous assignment. [e] Average values.

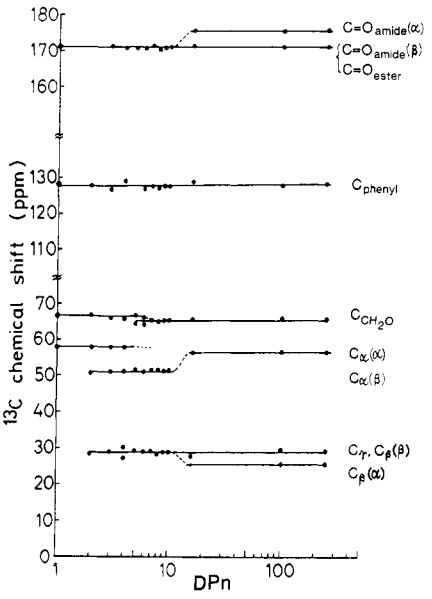


Figure 2. Plots of ^{13}C chemical shift of $[Glu(OBzl)]_n$ vs. DP_n.

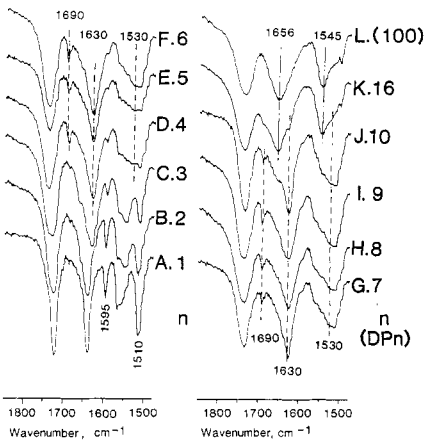


Figure 3. Infrared spectra of $[Glu(OBzl)]_n$ of various chain lengths. KBr disk, 1500–1800-cm^{-1} region. Alphabetical labelings of the samples are the same as in Figure 1.

In spite of the overlap of the methyl ^{13}C peak with the C_α peak of the β-sheet conformation and of the ester C=O peak with the amide C=O peak of the β-sheet conformation, respectively, it is possible to estimate the relative contribution of the α-helix and β-sheet forms from the following equations:

For the C_α peak

helix content (%) =

$$100(\text{peak } \alpha)(1 + X_M)/[(\text{peak } \alpha) + (\text{peak } \beta)] \quad (1)$$

where X_M is the M composition in the sequential copolymers ($0 \leq X_M \leq 1$).

For the C=O peak

helix content (%) =

$$200(\text{peak } \alpha)/[(\text{peak } \alpha) + (\text{peak } \beta)] \quad (2)$$

We emphasize that these estimates are correct only when both C_α and methyl groups (or both amide and ester carbonyl groups) give rise to equal peak intensities and are correct within the experimental error ±10% (or ±15%). It is clear from the above argument that the copolymers whose B composition is larger than 50% assume an α-helix conformation regardless of their sequences. On the other hand, copolymers in which M predominates assume a β-sheet conformation, as is evident from Figure 5. However, the following points should be taken into account. First, the effect of molecular weight is also important because the $\alpha(BMBM)_n$ (higher molecular weight fraction by gel filtration on Sephadex G-50) assumes the α-helix form, while the $\beta(BMBM)_n$ (lower molecular weight fraction) assumes the β-sheet form. Second, the manner of sample preparation also strongly influences the conformation, as seen for $(MBB)_n$ and $(BMMM)_n$. It is seen from spectra I and J of Figure 5 that the α-helix content is increased from 35% to 85% when the $(BMMM)_n$ sample

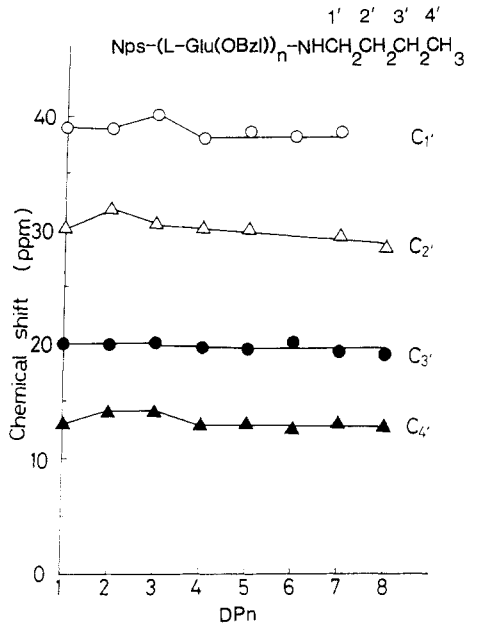
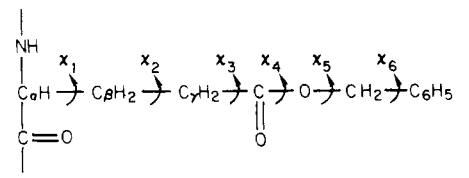


Figure 4. Plots of ^{13}C chemical shift of *n*-butylamide at the C-terminal moiety of oligo(γ-benzyl L-glutamates) vs. DP_n.

is dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and precipitated by diethyl ether. Such an effect is less significant for $(MMB)_n$ but increasing α-helix content is still recognizable.

Conformational features as revealed by the ^{13}C CP–MAS NMR spectra are generally consistent with findings by infrared spectra, although some disagreements are evident. As shown in Figure 7, characteristic bands for the α-helix are 1656 and 1545 cm^{-1} and those of the β-sheet form are 1630 and 1530 cm^{-1}. Closer examination of the relative peak intensities of the infrared spectra shows that $(MBBB)_n$, $(BBM)_n$, and $(MMBB)_n$ mainly adopt the α-helix form, with smaller amounts of the β-sheet form also present, consistent with the NMR data presented above. However, it appears that the relative proportions of the α-helix in $(MMB)_n$ and $(BMMM)_n$ are much higher than those observed in the ^{13}C CP–MAS NMR spectra. Further, a considerable amount of the β-sheet form is seen for $(M)_n$, although very little is seen in the CP–MAS NMR spectra. Such a discrepancy between the two methods is probably due to the manner of sample preparation, which may strongly influence the resultant conformation. It is likely that the higher proportion of β-sheet for $(M)_n$ observed by infrared spectra could be partly accounted for by a conformational change during sample preparation caused by mechanical shear. This view is consistent with previous data of Frushour and Koenig[29] and also our unpublished results on poly(L-alanines) (see footnote 36 of ref 2). Mechanical rolling increased considerably the amount of the β-sheet conformation for $(Ala)_n$.[29] Consistent with this, the infrared spectrum of $(M)_n$ film cast from dichloroacetic acid/diethyl ether shows α-helix bands only (Figure 7I). For this reason, we must be careful in interpreting infrared spectra taken for samples whose conformation can be easily changed by mechanical shear, such as in the preparation of the KBr disk. We emphasize that the ^{13}C NMR method is intrinsically nondestructive and convenient for quantitative purposes.

Line Widths. $[Glu(OBzl)]_n$ is a typical polypeptide with longer side chains. The conformational angles of the side-chain moiety can be characterized by angles χ_1 and χ_2 for $C_\alpha H–C_\beta H_2$ and $C_\beta H_2–C_\gamma H_2$ rotations, respectively. Further sets of angles, χ_3, χ_4, χ_5, and χ_6, are required to characterize the conformational features of the side chains:



It is expected that the ^{13}C chemical shifts of the side-chain moiety give rise to sharp signals comparable to those of the backbone moiety, if those conformational angles are fixed within certain narrow ranges. This is the case for polypeptides with shorter hydrophobic side chains such as $(Ala)_n$, $(Leu)_n$, $(Val)_n$, and $(Ile)_n$.[1,2,6,9] On the other hand, it appears that the line widths of the side-chain moiety of $[Glu(OBzl)]_n$ are increased substantially compared with those of the backbone moiety (Figure 1). This is obviously caused by the superposition of slightly different ^{13}C chemical shifts arising from distribution of the conformational angles in the side chains.[30,37] In this interpretation, we assume that various side-chain conformations are stable and are not rapidly interconverting on the NMR time scale, i.e., comparable to the frequency of the decoupling field.[40] In this connection, it is of interest to compare the ^{13}C line widths of higher molecular weight polymers (Figure 1M,L) with those of the 16-mer (Figure 1K). The line widths of the latter are very broad compared with those of the former, although their ^{13}C chemical shifts are the same. Our view is that such line broadening could be explained by the presence of a distribution of the conformational angles in the side-chain moiety, because of packing with lower crystallinity of the side chains. However, further work is necessary to ascertain whether such disordered side-chain moieties stem from a static distribution of conformers or rapid interconversion of these conformers.[41] It appears in this connection that the orientation of the side-chain moiety does not play a crucial role in stabilizing the α-helix conformation. Nevertheless, it is of particular interest to compare the ^{13}C line widths of the helical $(B)_n$ (Figure 1M,L) with those of helical $(M)_n$ (Figure 5K). The C_β and C_γ signals of the latter are much broader than those of the former. It is plausible that such disordered side-chain moieties of $(M)_n$ would destabilize the α-helix conformation. This view is supported by our finding that the α-helix conformation of $(M)_n$ is partly converted to the β-sheet form during preparation of the KBr disk, as observed by infrared spectra. On the contrary, no conformational change was induced for the much more stable α-helix of $(B)_n$. Therefore, it is concluded that the benzyl moiety of the Glu(OBzl) residue has, to some extent, an important role in stabilizing the α-helix conformation, as compared with the methyl group, in spite of the conclusion described above.

The ^{13}C NMR signals of the side chain, especially those of phenyl and CH_2O, have very broad line widths in the oligomers with DP_n between 5 and 10, which assume the β-sheet conformation (up to twofold broadening compared with the higher molecular weight polymers assuming the α-helix conformation). Thus, it is clear that there appears a distinction between the α-helix and β-sheet conformations as to the manner of orientation of the side-chain moiety. This is true for the copolymers taking the β-sheet conformation (Figure 5). Further, we found that the ^{13}C signals (C_β, CH_2O, and phenyl) of the side-chain moiety of poly(β-benzyl L-aspartate) taking the β-sheet conformation are also broadened to some extent because of the irregularity of side-chain order.[4] Therefore, it is clear that the benzyl side chains are considerably disordered in the β-sheet conformation. Accordingly, we emphasize that the ^{13}C NMR spectra of solid-state polypeptides provide an

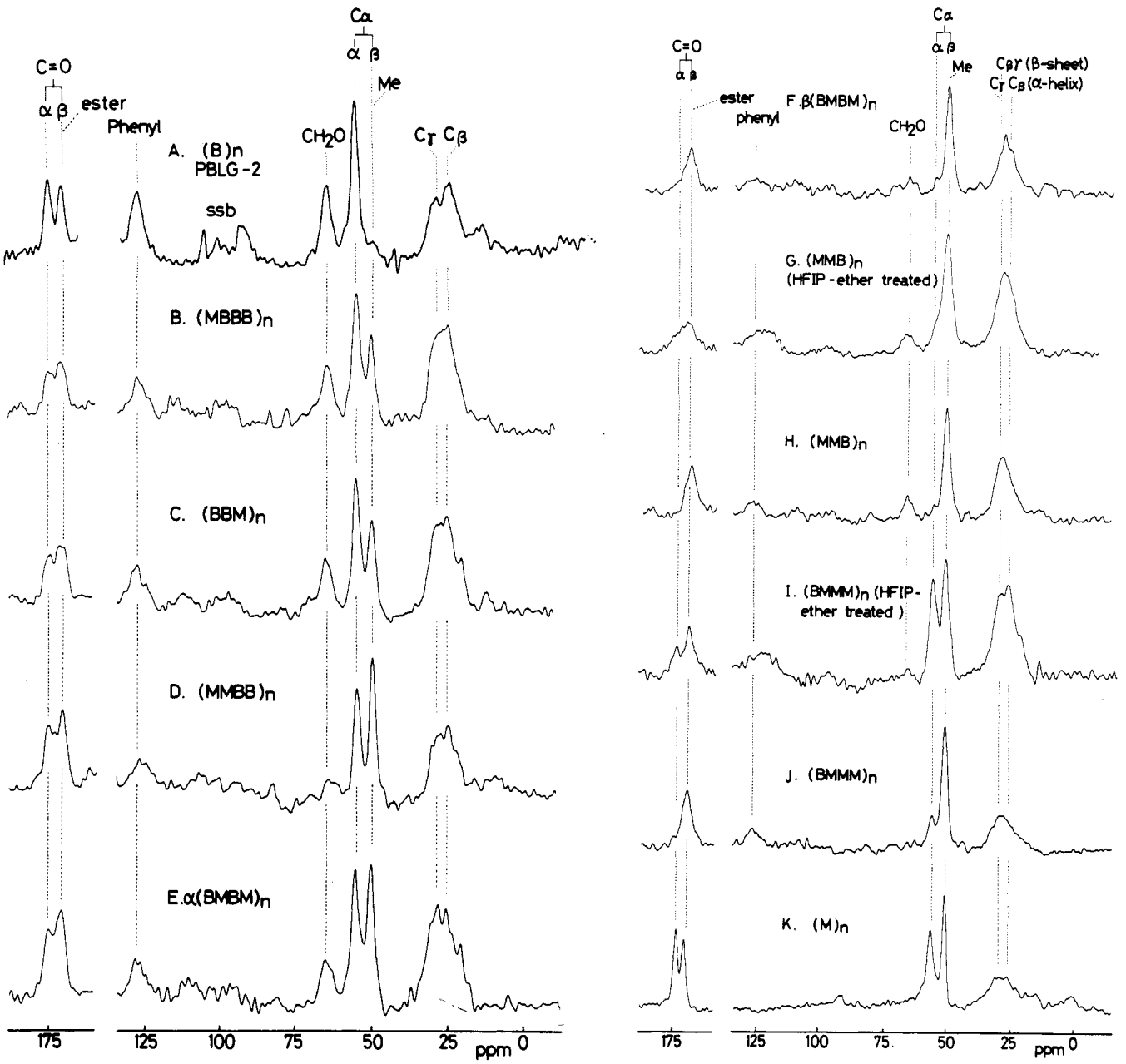


Figure 5. 75.46-MHz ^{13}C CP-MAS NMR spectra of the sequential copolymers $(B, M)_n$ consisting of γ-benzyl and γ-methyl L-glutamates in the solid state.

excellent means to obtain information regarding the orientation of their side chains.

Conformation-Dependent ^{13}C Chemical Shifts. As described above, there appears no significant change of the ^{13}C chemical shifts between the sequential copolymers and homopolymers including the oligomers, taking similar conformations. Therefore, the conformation-dependent ^{13}C chemical shifts of the backbone Glu moiety are effectively determined by their local conformations. It is interesting to compare the conformation-dependent ^{13}C chemical shifts of $[Glu(OBzl)]_n$ with those of other polypeptides studied so far, as summarized in Table II. As pointed out previously,[2] those polypeptides are classified into two groups: $(Ala)_n$ and $(Leu)_n$, and $(Val)_n$ and $(Ile)_n$ as viewed from the magnitude of the conformation-dependent ^{13}C chemical shifts of the α-helix relative to those of the β-sheet form, Δ. The latter group is characterized by larger $|\Delta|$ values in the C_α region but smaller $|\Delta|$ values in the carbonyl region. As mentioned previously,[2] such a change is caused by a smaller difference in the crystalline structure. In this connection, although the conformational characteristics of peptide residues with a β-CH_2 in the side chain, such as Ala, Leu, and Glu(OBzl), are known to differ from those with branched β-carbons, such as Val and Ile,[42] it is interesting to note that the conformation-dependent ^{13}C chemical shifts of not only the C_β in the side chain but also the C=O and C_α in the main chain for $[Glu(OBzl)]_n$ are classified in the group of $(Ala)_n$ and $(Leu)_n$ but not the group of $(Val)_n$ and $(Ile)_n$ in which the C_β carbon is disubstituted.

It is now feasible to examine whether or not the α-helix conformation present in solution is identical with that observed in the solid state. This comparison provides a clear picture of the stability of the α-helix in solution.[27,28,43-45] We found that ^{13}C chemical shifts of the α-helix in 3% TFA/97% $CDCl_3$[27] are in good agreement with the values summarized in Table II, except for deviation in the amide C=O signal (2.5 ppm).[46] This finding supports the view that the α-helix conformation of $[Glu(OBzl)]_n$ in solution is very stable as viewed from the NMR time scale, and there is no averaging process of chemical shifts due to rapid conformational fluctuation as seen for

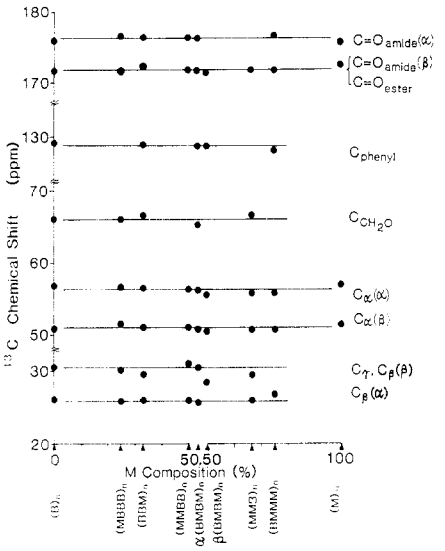


Figure 6. Plots of ^{13}C chemical shift vs. the composition of γ-methyl L-glutamate (M) in the sequential copolymers $(B, M)_n$.

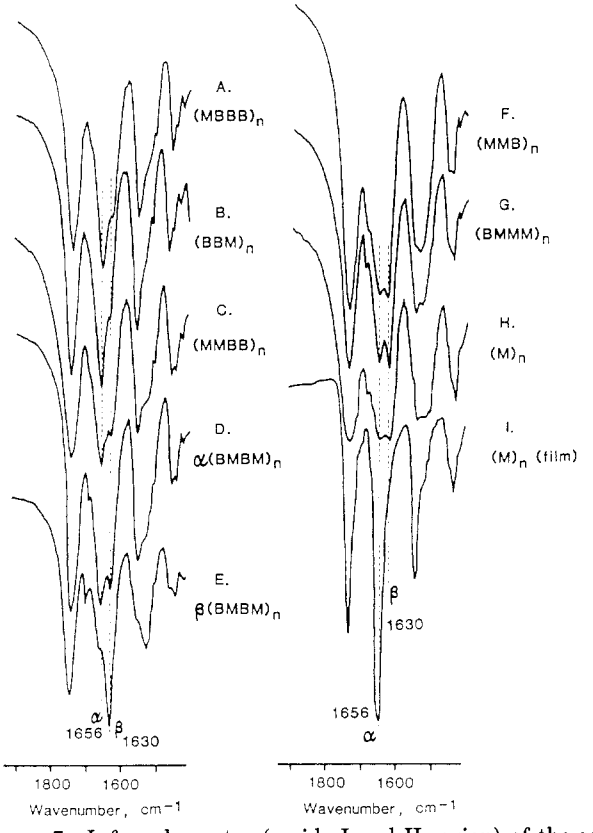


Figure 7. Infrared spectra (amide I and II region) of the sequential copolymers $(B, M)_n$ in the solid state (A–H, KBr disk) and its crystalline modification depending on sample preparation (I, film cast from dichloroacetic acid/diethyl ether).

helical amylose in aqueous solution[31] and also for poly(β-benzyl L-aspartate) in TFA/$CDCl_3$.[4,47] The discrepancy in the carbonyl group chemical shift is also worthy of detailed analysis, which will be published shortly.

It is well recognized that the *absolute* ^{13}C chemical shifts of the C_α and C_β signals (^{13}C chemical shifts of C_α and C_β are in the range 48–66 and 15–44 ppm, respectively) are strongly affected by the chemical structure of the individual amino acid residues and can be used effectively for microconformational studies of particular amino acid residues in peptides and proteins. On the other hand, C=O chemical shifts do not seem to be strongly affected by residue structure and could be used for diagnostic purposes of the main-chain conformation. In particular, it is obvious from Table II that the amide carbonyl ^{13}C chemical shifts for the α-helix conformation are in the range of 175.8 ± 0.8 ppm and those for the β-sheet form are in the range of 170.9 ± 1.2 ppm.[2,9]

Concluding Remarks

It is obvious from the foregoing discussion that the ^{13}C chemical shifts of solid $[Glu(OBzl)]_n$ and $(B, M)_n$ can be used as excellent conformational probes. Conformational features of the ^{13}C NMR spectra are generally in good agreement with those deduced from infrared spectra, but in some instances disagreements exist because of sample treatment for the infrared spectra. The role of the side-chain moiety in stabilizing the α-helix conformation can be analyzed by examination of the conformation of the sequential copolymer together with careful evaluation of the differential changes of the line widths. In particular, ^{13}C signals of the less ordered side-chain moieties exhibited considerable broadening. Therefore, careful evaluation of the line widths provides a clue to understanding the side-chain orientation.

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Registry No. Nps-[L-Glu(OBzl)]$_n$-NHBu (SRU), 90696-57-4; H-[L-Glu(OBzl)]$_n$-OH (homopolymer), 25014-27-1; H-[L-Glu(OBzl)]$_n$-OH (SRU), 25038-53-3; H-[L-Glu(OMe)]$_n$-OH (homopolymer), 25086-16-2; H-[L-Glu(OMe)]$_n$-OH (SRU), 25036-43-5; $(MBBB)_n$ (homopolymer), 90696-46-1; $(MBBB)_n$ (SRU), 90696-58-5; $(BBM)_n$ (homopolymer), 90696-48-3; $(BBM)_n$ (SRU), 90696-59-6; $(MMBB)_n$ (homopolymer), 90696-50-7; $(MMBB)_n$ (SRU), 90696-60-9; $(BMBM)_n$ (homopolymer), 90696-52-9; $(BMBM)_n$ (SRU), 90696-61-0; $(MMB)_n$ (homopolymer), 90696-54-1; $(MMB)_n$ (SRU), 90696-62-1; $(BMMM)_n$ (homopolymer), 90696-56-3; $(BMMM)_n$ (SRU), 90696-63-2.

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(30) This interpretation is based on a view that the ^{13}C chemical shift of *any* carbon exhibits a conformation-dependent change defined by the torsion angles about single bonds, although the extent of displacement of shifts could be varied in individual situations, e.g., variation of the torsion angle or functional groups attached. Previously, we found several kinds of conformation-dependent ^{13}C chemical shifts for the rotation about a single bond.[31-33]
In accordance with this expectation, we recently found that the CH_2O signals of the benzyl group are displaced by as much as 2.6 ppm, depending on amino acid composition as well as crystalline forms for a number of cyclic dipeptides containing L- or D-Glu(OBzl) group(s) (unpublished finding). Clearly, such significant displacement is caused by the presence of particular conformations defined by the χ_5 and χ_6 angles. Further, there appears a slight upfield displacement of the CH_2O signals (1.5 ppm) in going from the oligomers with $DP_n \leq 4$ to those with $DP_n \geq 5$, as indicated in Figure 1. Therefore, line broadening occurs when several conformers with various conformational angles are present, as in samples of lower crystallinity or amorphous materials.[31,32,34-36]
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Molecular Motion in Polycarbonates by Dipolar Rotational Spin-Echo ^{13}C NMR

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ABSTRACT: Molecular motions in polycarbonates and polycarbonate-like materials have been characterized by dipolar rotational spin-echo ^{13}C NMR. The dominant motion in polycarbonate is 180° flips about the aromatic-ring C_2 axes. These flips occur over a broad range of frequencies extending to over 15 MHz. The flips are superimposed on 30° ring oscillations about the same axes. Other main-chain motions, as measured through methyl-carbon dipolar patterns, are also significant; amplitudes of these motions are of the order of 20°. Chlorine substitution on the rings abolishes both ring and main-chain motions. Chemical modification of the links between rings also reduces motion, in some cases by preventing a fraction of the rings from flipping.

Introduction

The frequency of some of the main-chain motions of various polycarbonates has been determined from ^{13}C spin–lattice relaxation measurements employing high-resolution techniques, including magic-angle spinning.[1] In addition, the cooperative nature of main-chain motions has been inferred from the mechanical loss spectroscopy of an extended series of chemically substituted and modified polycarbonates and polycarbonate-like materials.[2] Despite the insights gained from such studies, to make an unambiguous connection between the macroscopic mechanical properties of these polymers and the microscopic molecular motions from which such properties derive, the amplitudes of the various ring and main-chain motions must be specified.

In this paper we measure the dipolar coupling between carbons and directly attached protons in polycarbonates using dipolar rotational spin-echo ^{13}C NMR.[3] The reduction in dipolar coupling by molecular motion (of frequency comparable to or greater than the dipolar coupling of about 10 kHz) becomes a measure of the amplitude of the molecular motion. Because this experiment is performed at natural abundance, it is practical to make such measurements on a series of polycarbonates, thereby aiding

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