the pentads -SSSSS- and -RSSSS- should be upfield with increased populations for 7′ and 8′, respectively. The observations in the 3′,4′ region for polymer VII are not dependable due to the irregularities present. Thus, our assignment scheme for the -SR- dyad region can only be properly tested with purer polymer of higher optical activity.

Finally, it should be mentioned that the H_4 methyl protons show triad effects with three signals, 1:2:1 being observed for polymer VI at 0.9 ppm.

Conclusion

The study of α,α-disubstituted poly(β-propiolactones) with high-field NMR has allowed us to obtain detailed information on the microstructure of the polymer chain.

Polymers prepared by anionic initiation starting from a racemic mixture of monomer have a random distribution of configurational units *R* and *S* in the chain. This was substantiated by a 100.6-MHz ^{13}C NMR analysis, for which an equal distribution of triads for stereosensitive carbon atoms was found. This rules out a stereoregular structure of the type of -RRSS-, which would be consistent with a 0.50:0.50 dyad distribution but not with the results of triad analysis.

The triad analysis performed on enantiomerically enriched polymers fits satisfactorily to Bernoullian distribution.

The most stereosensitive carbons are the methylene carbon C_3 in the main chain and the side-chain methyl carbon C_4.

Very similar and even more detailed results were obtained with 500-MHz ^1H NMR spectroscopy. For the α-methyl-α-*n*-propyl-substituted polymer tetrad effects with some pentad splittings were observed on methylene protons H_{3A} and H_{3B}, the latter being more stereosensitive than the former. Tetrad contents were calculated for polymers of various enantiomeric compositions according to Bernoullian statistics and good agreement with experimental results was found.

An assignment scheme for H_{3A} and H_{3B} tacticity effects on ^1H chemical shifts has been proposed. The ^1H NMR data for *n*-ad populations (Bernoullian) as a function of the enantiomeric enrichment parameter $p = (S)/(R)$ allow confirmation of the scheme out to the pentad level in the -SS- dyad. For the -SR- dyad two possible assignments schemes are consistent with the data at the tetrad level. Unequivocal assignment of the -RSRS- tetrad is necessary to remove this ambiguity.

Polymers prepared with zinc-coordinated initiators show up to 10% irregularities in structure due to side reactions (not yet characterized). For PMEPL using achiral initiator 1:2 $ZnEt_2$–MeOH almost no stereoselection occurred. For PMPPL using chiral initiator 1:1 $ZnEt_2$–DMBD a slight enrichment of the isotactic tetrad (ca. 7%) was observed.

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Secondary Structure of Peptides. 3. ^{13}C NMR Cross Polarization/Magic Angle Spinning Spectroscopic Characterization of Solid Polypeptides

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ABSTRACT: Natural-abundance 75.5-MHz ^{13}C NMR spectra of various solid polypeptides were measured by using the CP/MAS technique. The polypeptides were prepared by polymerizations of amino acid *N*-carboxyanhydrides under various conditions. The 75.5-MHz ^{13}C NMR CP/MAS spectra allow one to determine qualitatively and quantitatively α-helices in the presence of the antiparallel pleated sheet structure (or vice versa), to determine the poly(glycine II) structure (3_1 helix) in the presence of the poly(glycine I) structure (pleated sheet), and to distinguish the poly(proline I) structure (10_3 helix) from that of poly(proline II) (3_1 helix). A spectroscopic rule of general validity was found stating that in the case of CO and α-carbons the pleated sheet structure absorbs upfield of the α-helices, while it absorbs downfield in the case of β-carbons. The signals of γ-, δ-, and ε-carbons are not sensitive to a change of the secondary structure, except in the case of polyproline. A comparison of various polypeptides having identical secondary structures revealed that the chemical shifts of individual carbons obey the same substituent effects as in solution.

Introduction

In the past 5 years, ^{13}C NMR CP/MAS spectra have proved to be useful for the characterization of glassy[1-4] or partially crystalline polymers.[5-7] Crystalline and glassy areas of the same polymer may exhibit signals of different chemical shift and different line shape. Furthermore,

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investigations of various relaxation processes allow one to extract information on the segmental mobility in the solid state.[2,8,9]

In the preceding paper[10] we demonstrated for polyalanine, polyglycine, and polyvaline that ^{13}C NMR CP/MAS spectra also allow one to distinguish two different crystalline secondary structures, namely the α-helix and the antiparallel pleated sheet structure. Furthermore, we could demonstrate that the signal intensities are proportional to the mole fractions of the two secondary structures. Thus ^{13}C NMR CP/MAS spectra allow the quantitative determination of different crystalline secondary structures, and in this respect the NMR method seems to be significantly advantageous over X-ray diffraction and IR spectroscopy. The present work was undertaken first to elucidate whether all common polypeptides allow an NMR spectroscopic characterization of different secondary structures and second to establish chemical shift/primary structure and chemical shift/secondary structure relationships.

Experimental Section

Polypeptides. Poly(L-tryptophan), poly(L-histidine), poly(L-serine), and poly(L-4-hydroxyproline) were purchased from Sigma Chemicals (München/St. Louis).

α-Amino acid *N*-carboxyanhydrides (NCAs) were prepared by phosgenation of amino acids and twice recrystallized as described previously.[11] A typical primary amine initiated polymerization was conducted as follows. NCA, 40 mmol, was dissolved in 80 mL of acetonitrile (dried by distillation over P_4O_{10}); 1 mmol of benzylamine was added in the form of a 1 M solution in dry dioxane, and the flask was stoppered with a freshly prepared calcium chloride drying tube. After 2 days the polypeptide was precipitated from 400 mL of diethyl ether and dried at 70 °C (12 mm).

In the case of polyglycine 2 g of a sample prepared from 50 mmol of Gly-NCA and 1 mmol of benzylamine in acetonitrile were dissolved in 30 mL of a warm concentrated solution of LiBr and LiCl in water. This solution was dialyzed against water for 4 days; the precipitated polyglycine was filtered off, washed with water and methanol, and dried at 70 °C (12 mm). In the case of poly(L-proline), 2 g of a sample prepared from 50 mmol of L-Pro-NCA in 100 mL of dry pyridine at 20 °C was dissolved in 15 mL of formic acid, stored at room temperature for 1 day, and precipitated from 250 mL of diethyl ether.

Measurements. All 75.46-MHz ^{13}C NMR CP/MAS spectra were obtained on a Bruker CXP-300 FT spectrometer. Samples of 150–250 mg were measured in 6.3-mm i.d. rotors made of deuterated PMMA at spinning rates between 3.9 and 4.4 kHz. CP/MAS was applied with alternation of the 90° pulse phase. The proton pulse length was 4.5 μs, corresponding to an H_1 field strength of 58 kHz, which was found to be adequate for this class of compounds. A contact time of 3 ms and a repetition time of 4 s were used as "standard conditions". The standard conditions were found by systematic variation of both contact and repetition times in a number of characteristic cases (see ref 10 and later parts of this series). The magic angle was checked with glycine between the measurements; the line width of the carbonyl signal never exceeded 30 Hz. The chemical shifts were referenced to Me_4Si in the following way. The carbonyl signal of solid glycine was used as tertiary standard (170.09 ppm) and referenced to solid adamantane. The shift of solid adamantane was set equal to that of an adamantane solution in chloroform containing Me_4Si. In the case of polytyrosine and poly(L-tryptophan) the side bands were removed by means of the Dixon pulse sequence.[12] In all other cases the side bands were not removed; yet their shifts were varied by means of the spinning rate. Spinning rates >4.2 kHz are required to avoid overlapping of aromatic side bands with peptide CO signals of α-helices. Spinning rates <4.5 kHz are required to avoid overlapping of aromatic side bands with signals of CH_2O groups. Depending on the density of the sample in the rotor, 200–800 transients were accumulated.

The 50.34-MHz ^{13}C NMR CP/MAS spectra of Figure 1A,B were measured on a Bruker CXP-200 under the conditions mentioned above but at spinning rates of ca. 3.0 kHz. In both cases 1000 transients were accumulated. As expected the spinning side bands are considerably less intense than at 75.46 MHz. The better resolution compared to Figure 5B,C in ref 10 is due to a new probe head and a better dipolar proton decoupling.

The ^1H NMR end-group analyses were obtained with a Bruker WH-90 FT spectrometer in 5-mm o.d. sample tubes. Samples of 50 mg were measured with internal Me_4Si immediately after dissolution in 1 mL of deuterated trifluoroacetic acid (TFA). The signal intensities were determined by cutting out the peaks and weighing them.

Results and Discussion

α-Helix-Forming Difunctional Amino Acids. In the preceding paper[10] we reported that the signals of all three carbons of poly(L-alanine) are split into two peaks, one representing the α-helix (αh) and one representing the antiparallel pleated sheet (βs) structure. The intensity ratio of αh and βs peaks varied with the average degree of polymerization ($\overline{DP}$), so that increasing $\overline{DP}$s were associated with higher αh/βs intensity ratios. The simultaneous presence of α-helix and pleated sheet structure in various poly(L-alanine) and poly(L-leucine) samples prepared by primary amine initiated polymerizations of the corresponding amino acid NCAs has already been demonstrated by Kawai, Komoto, and co-workers by means of IR spectroscopy and X-ray diffraction.[13-15] Even though neither method allowed those authors an accurate quantification of both secondary structures, they could show that the helix content of $(L\text{-Ala})_n$ and $(L\text{-Leu})_n$ increases with increasing $\overline{DP}$s. The theoretical explanation given by Kawai et al.[13-15] is probably not correct, as discussed in the following paper of this series. However, the experimental fact that the α-helix content increases with the number average $\overline{DP}$ is highly useful for assignments of αh and βs peaks in the ^{13}C NMR CP/MAS spectra of all α-helix-forming polypeptides.

In the present work we have prepared a variety of α-helix-forming polypeptides by primary amine initiated polymerizations of the corresponding NCAs. This procedure was chosen because it allows one to vary the $\overline{DP}$s at will simply by varying the monomer/initiator ratio (eq 1).

$$\overline{DP} = \frac{[NCA]}{[amine]}\left(\frac{\%\ conversion}{100}\right) \quad (1)$$

The $\overline{DP}$s were checked by ^1H NMR end-group analyses as described in previous papers.[16,17] For this reason benzylamine was used as initiator for aliphatic monomers such as Ala-, Leu-, Nva-, or γOMe-Glu-NCA, whereas isopropylamine was used for Phe-NCA. We then assigned those peaks that showed increasing intensity with increasing $\overline{DP}$ to the α-helix structure as demonstrated for $(L\text{-Leu})_{10}$ and $(L\text{-Leu})_{20}$ in Figure 1. In order to check these assignments IR spectra were measured in KBr. As expected, all α-helix-forming polypeptides with $\overline{DP} > 20$ exhibited an amide I band with a maximum at 1660 ± 2 cm^{-1}, in agreement with an α-helical secondary structure. Further evidence for the reliability of our assignment was obtained by the investigation of poly(D,L-amino acids). Racemic NCAs of alanine, norvaline, leucine, methionine, and phenylalanine were polymerized in dioxane at 100 °C with benzylamine as initiator, so that $\overline{DP}$s = 10 were obtained. We have expected that the polypeptides formed under these conditions would be almost free of α-helices for two reasons. First, the low $\overline{DP}$s favor β-sheet structures and, second, the α-helix is destabilized by the incorporation of enantiomeric monomer units.[18] In previous papers dealing with the NMR spectroscopic tacticity analysis of

Table I
^{13}C NMR Chemical Shifts of Solid Polypeptides That Can Adopt the α-Helix Structure

poly-peptide	secondary structure	δ of amino acid residues[a]							
		CO	α-C	β-C	γ-C	δ-C	ε-C		
(L-Ala)$_n$	α-helix	176.8	52.8	15.5					
	β-sheet	172.2	49.3	20.3					
(α-Abu)$_n$	α-helix	175.8	59.6	23.7	10.4				
	β-sheet	172.0	53.8	28.5					
(Nva)$_n$	α-helix	175.9	55.5	33.3	19.0	14.0			
	β-sheet	172.3	52.6	37.2					
(Leu)$_n$	α-helix	175.8	55.8	43.7	24.1	24.1			
	β-sheet	171.3	51.2	39.6	25.6				
(Phe)$_n$	α-helix	175.2	61.3	35.0	138.4		127.6		
	β-sheet	169.0	53.2	39.3	136.3				
(Tyr)$_n$	α-helix	176.7	54.8–58.6	36.1	(129.7)			(116.1)	(154.2)
	β-sheet	169.6	52.1	39.3	(128.0)[b]			(115.0)[b]	(155.2)[b,c]
(Met)$_n$	α-helix	175.1	57.2	30.2	30.2		14.7		
	β-sheet	170.6	52.2	34.8	30.0		15.7		
(L-His)$_n$	β-sheet	170.8	53.2	29.8	115.3/134.9				
(L-Trp)$_n$	α-helix	174.6	58.6	27.2	135.3	125.4	119.6/110.3		

[a] Given in ppm relative to Me_4Si; margin of error ±0.3. [b] Assignments to helix and sheet structure are not clear. [c] Quart, carbon attached to OH.

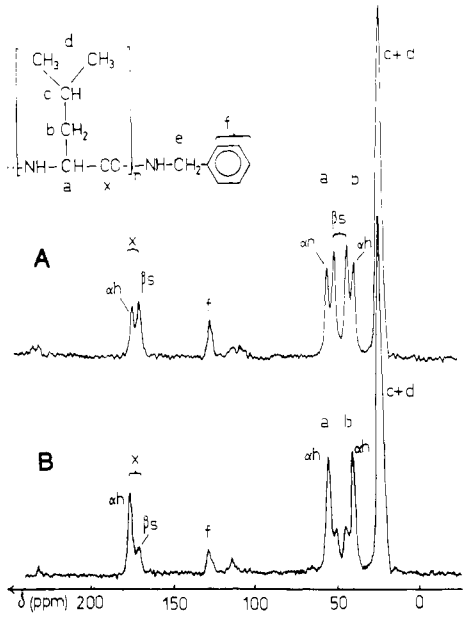


Figure 1. 50.4-MHz ^{13}C NMR CP/MAS spectra of (A) (L-Leu)$_{10}$ and (B) (L-Leu)$_{20}$ obtained by benzylamine initiated polymerizations of L-Leu-NCA in dioxane at 20 °C.

poly(D,L-amino acids),[17,19,20] we demonstrated that primary amino initiated polymerizations of D,L-NCAs at temperatures ≥ 100 °C yield nearly atactic poly(D,L-amino acids). Random stereosequences are in turn least favorable for the formation of α-helices. In agreement with these considerations the ^{13}C NMR CP/MAS spectra of the low molecular weight poly(D,L-amino acids) displayed only one set of peaks, which were nearly identical with the "βs" peaks of the corresponding poly(L-amino acids).

Furthermore, it is worth mentioning that the ^{13}C NMR signal pattern of polyalanines in TFA solution[21] resembles that of the ^{13}C NMR CP/MAS spectra. High molecular weight poly(L-alanine) is helical in pure TFA whereas oligomers and poly(D,L-alanines) adopt a random-coil conformation. In the case of the α-C and CO group the helix peaks absorb downfield from the random-coil peaks, whereas the helix peak of the CH_3 signal appears at higher field. Since the chemical shifts of dissolved peptides depend not only on their conformation but also on direct solvent effects (H bonds, protonation, and dipolar forces), it is not yet clear whether the similarity of solution and solid-state spectra is more coincidental or a rule resulting from the predominant influence of the conformation. However, the analogous signal pattern of solid poly(L-prolines) and of various *N*-acyl-L-prolines in organic solution (see below) suggests that the latter hypothesis is true.

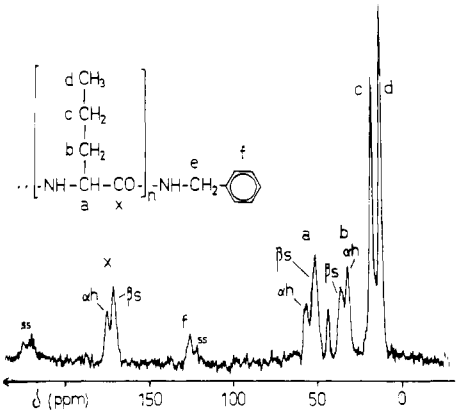


Figure 2. 75.4-MHz ^{13}C NMR CP/MAS spectrum of poly(L-norvaline) $\overline{DP}$ = 20, obtained by benzylamine initiated polymerization of L-Nva-NCA in acetonitrile at 20 °C.

The chemical shifts of all peptides derived from difunctional amino acids investigated so far are listed in Table I and those of α-helix-forming trifunctional amino acids in Table II. A comparison of the chemical shifts resulting from helix and β-sheet structures are consistent with the following spectroscopic rules: (I) In the case of carbonyl and α-carbons the pleated sheet structure absorbs upfield from the α-helices. (II) In the case of β-carbons the pleated sheet structures absorb downfield from the α-helices. (III) The signal of γ-, δ-, and ε-carbons are not sensitive to the secondary structure (Figure 2).

Concerning rule I, it is to be mentioned that the spectra of all α-helical polypeptides exhibit a CO peak at 176 ± 1 ppm while the carbonyl peak of the pleated-sheet structure shows up at 171 ± 1.5 ppm. The limits of the shift range of αh and βs peaks are mainly a consequence of substituent effects (discussed below). The margin of error is of the order of ±0.3 ppm and results from the large line width and from the influence of noise. Characteristic chemical shifts of the αh and βs peaks of the α-carbons cannot be extracted from the data of Table I because the α-carbon signals depend largely on the nature of the side chains (see below). The validity of rule III is, of course, dependent on the resolution and, thus, on further progress

Table II
^{13}C NMR Chemical Shifts of Solid Polypeptides Derived from Aspartic Acid, Glutamic Acid, Ornithine, or Lysine

polypeptide	secondary structure[a]	δ of amino acid residue[a] α-CO	α-C	β-C	γ-C	δ-C	ε-C	δ of protecting groups
$(Asp)_n$ Na salt	β-sheet	173.8	51.0	38.7	176.8			
$(\beta OBzl\text{-}Asp)_n$	α-helix	174.9	53.6	34.2	168.6			135.7, 128.2, 66.1
$(Glu)_n$ acid	β-sheet	171.9	52.7	29.9	29.9	174.8		
Na salt	β-sheet	172.6	51.3	30.9	30.9	180.0		
$(\gamma OMe\text{-}Glu)_n$	α-helix	175.9	47.0	26.3	29.7	172.0		51.1 (CH_3)
	β-sheet	172.2	51.2	29.8	29.8			
$(\gamma OBzl\text{-}Glu)_n$	α-helix	175.4	56.8	25.9	30.3	172.2		136.0, 128.0, 65.8
	β-sheet	172.2	51.1	29.7	29.7			
$(N^{\delta}\text{-HBr-Orn})_n$	β-sheet	173.3	53.7	28.1	24.5	40.1		
$iso(N^{\alpha}\text{-HBr-Orn})_n$	β-sheet	170.8	53.5	28.9	24.5	39.7		
$(N^{\delta}\text{-Z-Orn})_n$	α-helix	175.6	57.5	27.0	27.0	40.2		155.8, 136.4, 127.4, 65.5
$(N^{\delta}\text{-Tfa-Orn})_n$	α-helix	175.1	57.5	26.7	26.7	39.6		157.5, 115.7
$(N^{\epsilon}\text{-HBr-Lys})_n$	β-sheet	171.5	52.3	27.0	33.9	22.7	40.1	
$iso(N^{\alpha}\text{-HBr-Lys})_n$	β-sheet	169.1	53.7	24.8	31.2	24.8	40.4	
$(N^{\epsilon}\text{-Z-Lys})_n$	α-helix	175.7	57.6	29.3	29.3	23.6	40.6	156.5, 136.4, 127.9, 65.3
	β-sheet	170.4	51.4	28.5	28.5	22.2	40.2	155.4, 135.9, 126.7, 65.1
$iso(N^{\alpha}\text{-Z-Lys})_n$	β-sheet	171.0	54.8	30.4	24.4		39.1	155.8, 135.3, 127.5, 67.0

[a] Given as ppm relative to Me_4Si.

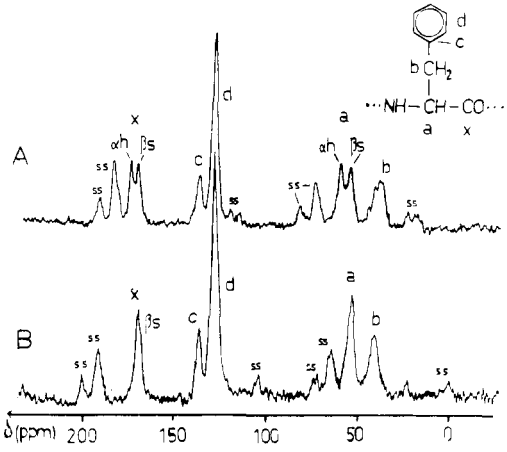


Figure 3. 75.4-MHz ^{13}C NMR CP/MAS spectra of (A) (L-Phe)$_{20}$ obtained by isopropylamine initiated polymerization of L-Phe-NCA in acetonitrile at 20 °C and (B) (L-Phe)$_{20}$ obtained by isopropylamine initiated polymerization of L-Phe-NCA in dioxane at 20 °C.

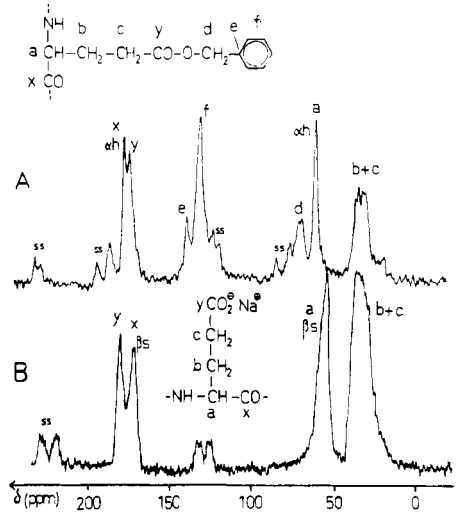


Figure 4. 75.4-MHz ^{13}C NMR CP/MAS spectra of (A) $(\gamma OBzl\text{-}Glu)_{20}$ obtained by isopropylamine initiated NCA polymerization in dioxane at 20 °C and (B) the sodium salt of poly(glutamic acid), $\overline{DP} \sim 50$.

in instrumentation. It is also noteworthy that the signals of the γ- and δ-carbons of $(L\text{-}Leu)_n$ and $(D,L\text{-}Leu)_n$ have a slightly different shape, indicating a weak sensitivity to a change of the secondary structure.

Finally it is to be noted that the intensity ratios of αh and βs peaks of $(L\text{-}Phe)_{20}$ varied significantly when L-Phe-NCA was polymerized with isopropylamine under various conditions (Figure 3). However, identical intensity ratios of αh and βs peaks were found when benzylamine initiated polymerizations of L-Leu-NCA (M/I = 20) were run in dioxane and acetonitrile at 20 °C. This means that the mole fraction of α-helices may depend also on the reaction conditions and not only on the $\overline{DP}$. Thus the sensitivity of the secondary structure to the reaction conditions may be different for various polypeptides. A more detailed investigation of this problem is in progress and will be published elsewhere.

α-Helix-Forming Trifunctional Amino Acids. The secondar structure of polypeptides of acidic or basic amino acids may vary with the nature of the substituents attached to their side chain. For instance, the β- or γ-esters of L-aspartic or L-glutamic acid are known to form α-helices in nonacidic solutions and in the solid state.[22] Accordingly, we found the expected two α-C signals of the β-sheet (51.1 ppm) and the α-helix structure (56.8 ppm) when the ^{13}C NMR of poly(γ-benzyl glutamate) of $\overline{DP}$ = 10 was measured (Table II). Samples with higher $\overline{DP}$s only exhibited the signal at 56.8 ppm in agreement with a helix content of nearly 100%. The ^{13}C NMR spectra of such samples display two carbonyl signals of equal intensity at 175.5 and 172.2 ppm, while a lower intensity of the downfield signal is observed for $\overline{DP} \leq 10$. Thus, we may attribute the downfield signal to the α-CO group in the helical state, while the upfield signal represents both carbonyls, the α-CO in a β-sheet environment and the γ-CO, which is insensitive to the secondary structure (Figure 3B). The spectroscopic behavior of poly(γ-benzyl L-aspartate) is similar to that of the glutamate, although it forms a left-handed helix, while $(\gamma OBzl\text{-}L\text{-}Glu)_n$ is a right-handed α-helix like other poly(L-amino acids) (Figure 4). The spectra of poly(γ-methyl L-glutamate) differ from those of $(\gamma OBzl\text{-}L\text{-}Glu)_n$ in the α-C region because the methyl signal, which is insensitive to the secondary structure, overlaps with the α-C signal of the β-sheet form. This means that samples with nearly 100% α-helix content display two signals of equal intensity at 56.8 and 51.1 ppm,

but only one signal at 51.1 ppm when helices are absent. Hence, quantification of the helix content requires eq 2 when the α-C peaks of $(OBzl\text{-}L\text{-}Asp)_n$ and $(OBzl\text{-}L\text{-}Glu)_n$ are measured, while it requires eq 3 for the evaluation on the carbonyl signals or the α-C signals of $(\gamma OMe\text{-}L\text{-}Glu)_n$.

$$\text{mol \% } \alpha h = \frac{I_{\alpha h} \times 100}{I_{\alpha h} + I_{\beta s}} \tag{2}$$

$$\text{mol \% } \alpha h = \frac{I_{\alpha h} \times 100}{\frac{1}{2}(I_{\alpha h} + I_t)} \tag{3}$$

I_t = total intensity of side-chain signal + overlapping main-chain (βs) signal.

In this connection we wish to emphasize that the correct quantification of the signal intensities requires, of course, a proper adjustment of the acquisition parameters. In the preceding paper we demonstrated for mixtures of poly(L-alanine) and poly(L-valine) or poly(L-alanine) and polyglycine that the intensity ratio of the CO signals is insensitive to a variation of the contact time, to a variation of the repetition time, and to a mismatch of the Hartmann–Hahn condition. This previous investigation on intensity ratios involved only signals of main-chain carbons. However, the quantitative evaluation of polyaspartate and polyglutamate spectra may involve a comparison of main-chain and side-chain carbons. Depending on the nature of the side chain and on the secondary structure the mobility of side-chain segments may differ more or less from the mobility of main-chain segments. Consequently the relaxation rates of the protons ($T\rho$) may depend on the nature of the side chain, so that both intensities and intensity ratios of the ^{13}C signals of polyaspartates and polyglutamates may vary considerably with contact time. A more detailed investigation of (γOMe-L-Glu) and (γOBzl-L-Glu) will be described in another part of this series. In the present work we only wish to report that the intensity ratio of the two carbonyl signals is rather insensitive to variations of the contact time within the limits of 0.8 and 3 ms. In the case of (γOBzl-L-Asp) and $(\gamma OBzl\text{-}L\text{-}Glu)_n$, 2 ms seems to be a suitable contact time for both α-C and CO signals. In the case of $(\gamma OMe\text{-}L\text{-}Glu)_n$ the optimum values are 1.0–2.0 ms for the CO signals and 0.5–0.8 ms for the aliphatic carbons.

In addition to γ-esters we have investigated the sodium salts of poly(L-aspartic acid) and poly(glutamic acid) along with the free poly(glutamic acid). The IR spectra of the three samples indicated that all three polypeptides had adopted a sheet structure. This result was expected for the sodium salts, which due to their positively charged side chains cannot adopt a helix structure. However, poly(glutamic acid) forms α-helices in H_2O at pHs <5, and thus, we expected the solid poly acid also to be helical. Perhaps the HBr treatment of $(\gamma OBzl\text{-}L\text{-}Glu)_n$, which we used to obtain the free acid, led to partial racemization, which may destabilize the α-helix structure. The ^{13}C NMR CP/MAS spectra of all three samples confirm the β-sheet structure, exhibiting only one α-C signal in the range 51.0–52.7 ppm, which agrees well with the values found for the β-sheet forms of $(\gamma OMe\text{-}L\text{-}Glu)_n$ and $(\gamma Bzl\text{-}L\text{-}Glu)_n$ (Table II). Furthermore, the spectra of all three samples display two carbonyl signals with the upfield signal in the range 171.9–172.6 ppm. Because this shift range is typical for β-sheet structures, we have assigned the upfield signal to the peptide carbonyl. This assignment leads to an inverse signal pattern when the salts and esters of poly(L-glutamic acid) are compared (Figure 4A,B). However, it is confirmed by the observation that the γ-carbonyl of sodium polyglutamate absorbs downfield from the free

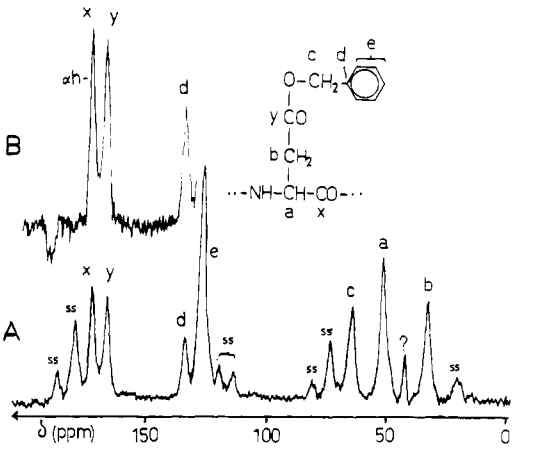
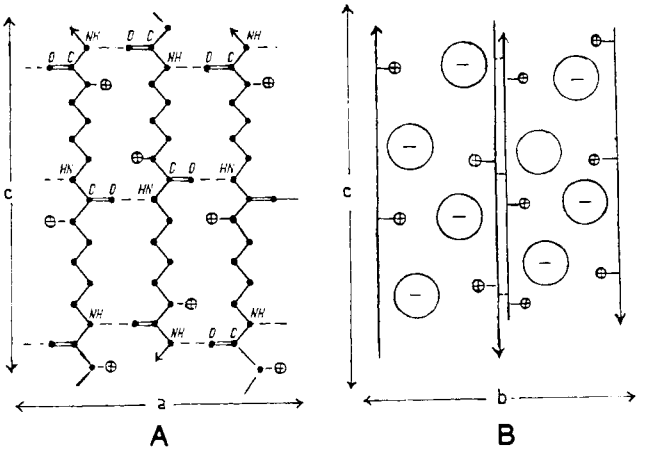


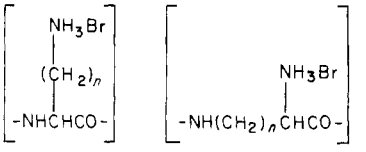
Figure 5. 75.4-MHz ^{13}C NMR CP/MAS spectrum of poly(β-benzyl L-aspartate) obtained by pyridine initiated NCA polymerization at 20 °C: (A) normal spectrum; (B) nonprotonated carbons only.

Scheme I
View on the *c*/*a* Plane (A) and the *c*/*b* Plane (B) of the Hypothetical Crystal Lattice of Isopoly(N^{ϵ}-HBr-L-lysine)



acid. A downfield shift upon dissociation is the characteristic spectroscopic behavior of all COOH groups.[24]

In the case of poly(L-ornithine) and poly(L-lysine), of course, the protonated forms, with positively charged side chains, normally do not adopt the helix structure. We have confirmed by IR spectra that the N^{α}-hydrobromides **1a,b**



1a,b **2a,b**

a, n = 3; b, n = 4

exist in the pleated sheet structure and α-C and CO signals of the ^{13}C NMR CP/MAS spectra show the corresponding chemical shifts (Table II). It is noteworthy that all aliphatic carbons give well-resolved signals, in contrast to the isopolypeptides **2a,b**, so that the ^{13}C spectra allow a clear distinction (Figure 5A,B). Originally, we expected better resolved spectra for the isopolypeptides, it being assumed that the aliphatic chains are exposed to a nearly alternating pattern of positive and negative charges (Scheme IB). The isopolypeptide hydrobromides **2a,b** have been previously prepared by us,[25,26] and X-ray diffraction analyses of their crystal lattice do not yet exist. Since they may be considered as substituted nylons, it is not unlikely that they

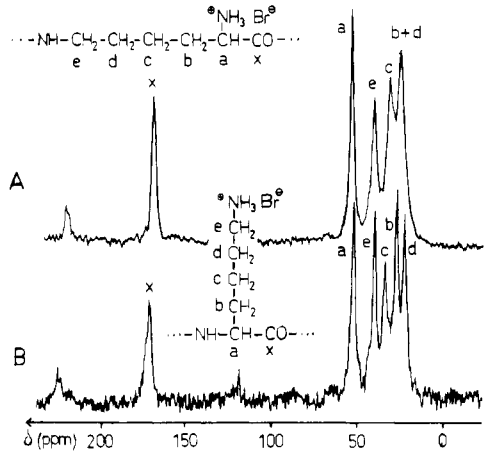


Figure 6. 75.4-MHz ^{13}C NMR CP/MAS spectra of (A) iso-poly(N^{α}-HBr-L-Lys) and (B) poly(N^{ϵ}-HBr-L-Lys).

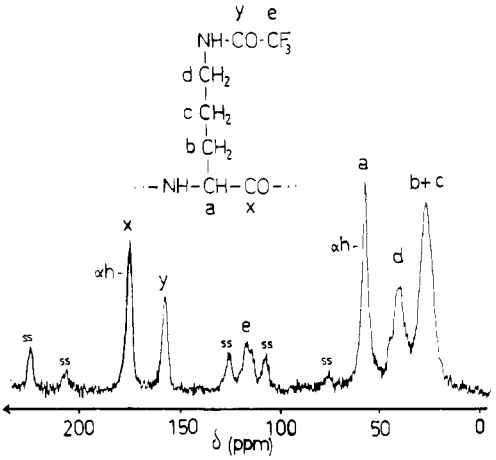


Figure 7. 75.4-MHz ^{13}C NMR CP/MAS spectrum of poly-(N^{δ}-Tfa-L-Orn) obtained by pyridine initiated NCA polymerization at 80 °C.

form H-bond networks similar to Nylon-5 and Nylon-6 (Scheme IA). The main difference would then be the lateral packing of the bonded sheets, which are separated (but also cross-linked) by a nearly regular array of positive and negative ions. We have now measured X-ray powder patterns of **2a** and **2b** samples crystallized from water (ethanol) ether. Unfortunately, only an intense halo without fine structure was found, which did not allow a clear decision whether the structure proposed in Scheme I is correct.

Furthermore, it is noteworthy that the carbonyl groups of the isopolypeptides **2a,b** absorb upfield from those of the polypeptides **1a,b** (Table II, Figure 6). A similar shift effect is observable in water solution, demonstrating the the substituent effect of the α-NH_3^+ group is operative in both solution and the solid state. Other substituent effects behave similarly (vide infra).

In contrast to the hydrobromides, the N^{ω}-benzyloxycarbonyl derivatives of poly(L-ornithine) and poly(L-lysine) give spectra similar to those of other helical polypeptides with carbons in the γ-position. We find the α-C signal near 57 ppm and the CO signal near 176 ppm. The urethane carbonyl is clearly separated from the peptide carbonyl and does not affect a quantitative evaluation of signal intensities. The same is true for the trifluoroacetyl group of poly(N^{δ}-Tfa-L-Orn) (Figure 7) by analogy with the spectroscopic behavior in solution.

3_1-Helix-Forming Polypeptides. In addition to an antiparallel pleated sheet structure (PGI), polyglycine can adopt a 3_1 helix (PGII) stabilized by interchain H

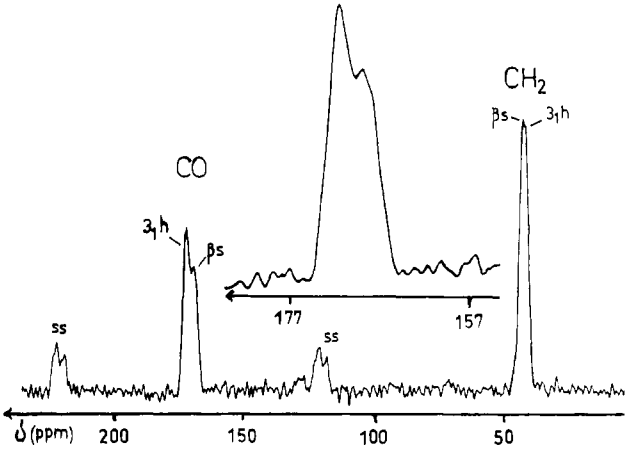


Figure 8. 75.5-MHz ^{13}C NMR CP/MAS spectrum of $(Gly)_{50}$ prepared by benzylamine initiated NCA polymerization in acetonitrile at 20 °C after dialysis from a concentrated LiBr + LiCl solution (insert: expanded CO signal).

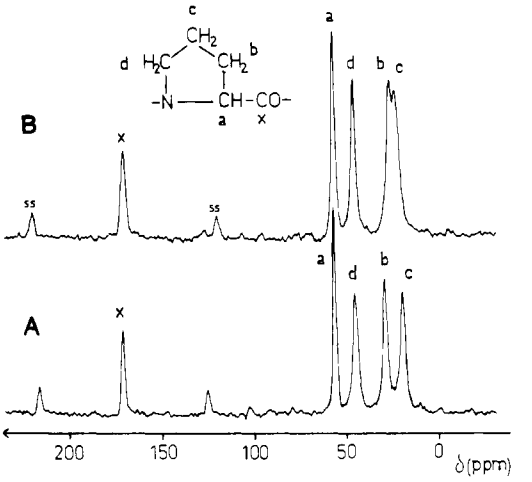


Figure 9. 75.4-MHz ^{13}C NMR CP/MAS spectra of (A) poly(L-proline I) (10_3 helix) obtained by NCA polymerization in pyridine at 20 °C and (B) the same sample after reprecipitation from formic acid/diethyl ether (3_1 helix).

bonds.[27,28] Polyglycine obtained by polymerization of Gly-NCA or by reprecipitation from acidic solution (e.g., TFA) possesses the sheet structure. Indeed, the 75.5-MHz ^{13}C NMR CP/MAS spectra of a sample with average DP = 50 prepared by benzylamine-initiated NCA polymerization in acetonitrile was unchanged after reprecipitation from TFA/methanol. The same sample was dissolved in a concentrated LiBr/LiCl solution and precipitated by dialysis to cause helix formation. The spectrum obtained in this way displays two additional signals that may be attributed to the 3_1 helix (Figure 8); yet the signals of the sheet structure have not completely disappeared.

Another class of 3_1-helix-forming polypeptides are poly(L-proline) and polypeptides of similar cyclic imino acids. It is known[29] that poly(L-proline) takes on the compact 10_3 helix (PPI structure) when L-Pro-NCA is polymerized in pyridine at room temperature. Precipitation from acidic solutions yields, after complete mutorotation, the 3_1 helix (PPII structure).[30,31] Samples prepared under these conditions gave two different ^{13}C NMR CP/MAS spectra (Figure 9A,B). Most characteristic are the shift differences of the β- and γ-carbons, which are considerably greater for the 10_3 helix (cis amine bonds) than for the 3_1 helix (trans amine bonds). Similar signal patterns are known from various N-acylproline derivatives in solution, provided the cis/trans equilibrium allowed the

Table III
^{13}C NMR Chemical Shifts of Polypeptides That Cannot Adopt the α-Helix Conformation

polypeptide	secondary structure	δ of amino acid residue[a] CO	α-C	β-C	γ-C	δ-C	δ of protecting groups
$(Gly)_n$	3_1 helix	172.1	42.0				
		168.9	42.9				
	β-sheet	169.2	44.3				
$(\beta\text{-Ala})_n$	β-sheet	170.5	33.3	35.7			
$(Val)_n$[a]	β-sheet	171.5	58.2	32.4	18.4		
$(Ile)_n$[a]	β-sheet	171.0	57.1	33.1	13.8	11.4	
$(Ser)_n$	β-sheet	170.0	54.6	62.3			
$(S\text{-Bzl-Cys})_n$	β-sheet	170.0	52.8	35.8			134.6, 128.1, 35.8
$(Phg)_n$	β-sheet	169.2	55.5	136.8	126.8		
$(Pro)_n$	10_3 helix	170.4	58.0	31.4	21.5	47.0	
	3_1 helix	169.6	57.5	27.3	24.4	46.5	
$(4\text{-Hpr})_n$	3_1 helix	171.2	57.6	55.8	70.5	36.5	
(L-thiazolidine-4-carboxylic acid)$_n$	3_1 helix	167.6	61.8	32.7		50.7	
$(Sar)_n$	?	168.5	49.2		34.9		

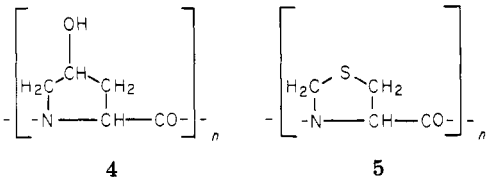
[a] Given as ppm relative to Me_4Si. [b] Chemical shifts were also reported in ref 35.

detection of both isomers.[32] Also, in solution, the shift difference between β-C and γ-C signals of the cis isomer is greater than of that of the trans isomer. Apparently, the conformation of the proline ring is mainly a consequence of the cis or trans configuration of the next neighbor (*N*-acyl residue), and the helical structure or the spatial arrangement of helices does not contribute much to it.

In addition to the polymerization of L-Pro-NCA in pyridine at 20 °C a benzylamine initiated polymerization was conducted in dioxane at 100 °C. Surprisingly the optical rotation measured immediately after dissolution in acetic acid had a value ($[\alpha]^{20}_D$ −230°) between the extremens of the 3_1 and the 10_3 helix. After prolonged standing, complete mutarotation was observed ($[\alpha]^{20}_D$ −540°), indicating that the initial value was not the result of partial racemization. Hence, it is obvious that the polymerization in dioxane at 100 °C led to a mixture of 10_3 and 3_1 helices. The ^{13}C NMR CP/MAS spectrum (Figure 10A) confirms this assumption, and resolution enhancement followed by quantification of the signal intensities indicates a composition of 60 ± 5% PPI form (10_3 helix) along with 40 ± 5% PPII. The quantitative evalution of signals after resolution enhancement requires that the signals under investigation are identical with respect to both line shape and line width. We have found that all signals of PPI and PPII possess a nearly perfect Lorentzian line shape, and at least the signals of the CH_2 groups also have identical line widths. Thus, our experiments demonstrate the the secondary structure of poly(L-proline) depends strongly on the reaction conditions used for the NCA polymerization.

We have measured spectra of poly(D,L-proline) obtained by pyridine initiated polymerization of D,L-proline *N*-thiocarbonic acid anhydride (**3**). ^1H, ^{13}C, and ^{15}N NMR spectra[33,34] measured in water or dimethyl sulfoxide demonstrate that dissolution of this stereocopolypeptide leads to thermodynamically controlled equilibria of cis and trans peptide bonds. In other words 10_3 and 3_1 helices are simultaneously present and a complete mutarotation was never observed. The ^{13}C NMR spectrum of Figure 10B demonstrates that in the solid phase trans amide bonds, i.e., 3_1-helix segments, prevail over cis amide bonds (10_3 helices) in analogy with water solutions.[33,34] However, additional signals at 45.4 and 18–20 ppm suggest that a third kind of secondary structure is present, which presumably originates from segments with an alternating sequence of L and D units.

Finally, we have listed the ^{13}C NMR data of poly(L-4-hydroxyproline) (**4**) and poly(L-thiazolidinecarboxylic acid) (**5**). Both polypeptides are known to adopt the 3_1-helix



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structure, because, for steric reasons, the more compact 10_3 helix is not accessible. Hence, we believe that the data listed in (Table III) represent 3_1 helices. We have also looked at the spectrum of polysarcosine, because this is the poly(imino acid) with the simplest chemical structure. To the best of our knowledge studies of its secondary structure have not yet been reported. We have observed that polysarcosine prepared from Sar-NCA in solution contains up to 3% solvent (e.g., dioxane or pyridine), which cannot completely be removed at temperatures <150 °C. Obviously, the solvent molecules are included in the crystal lattice and eventually contribute to its stabilization. We have compared three samples: (A) crystalline Sar-NCA polymerized at room temperature in the crystalline state by storage in air; (B) Sar-NCA polymerized in pyridine at 20 °C; (C) Sar-NCA polymerized by means of benzylamine in dioxane at 20 °C. All three samples gave identical ^{13}C NMR CP/MAS spectra, so that the influence of the solvent (or other reaction conditions) on the secondary structure was not detectable. Since polysarcosine lacks

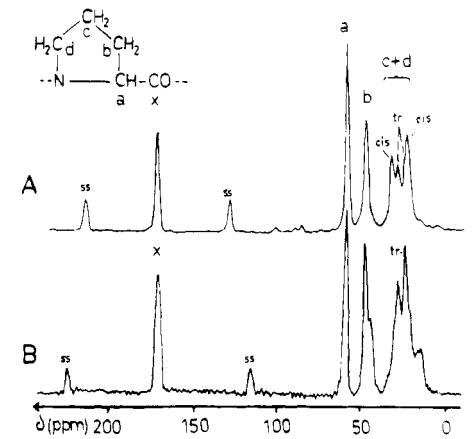

Figure 10. 75.4-MHz ^{13}C NMR spectra of (A) (L-Pro)$_{20}$ obtained by benzylamine initiated NCA polymerization in dioxane at 100 °C and (B) (D,L-Pro)$_{110}$ obtained by polymerization of D,L-proline *N*-thiocarbonic acid anhydrosulfide in pyridine at 20 °C.

Table IV
^{13}C NMR Chemical Shifts of Various Polypeptides Dissolved in a Mixture of TFA + 5% (by Volume) MSA

polypeptide	δ values					
	CO	α-C	β-C	γ-C	δ-C	ε-C
$(Gly)_n$	173.62	43.32				
$(Ala)_n$	176.20	51.48	16.18			
$(\alpha\text{-Aib})_n$	178.22	59.68	23.63			
$(\alpha\text{-Anb})_n$	175.58	57.23	25.19	8.79		
$(Val)_n$	174.93	61.55	31.23	17.91 17.48		
$(Nva)_n$[b]	175.74	55.83	33.66	18.83	12.03	
$(Leu)_n$	175.83	54.22	40.25	24.98	21.47 20.34	
$(Phg)_n$[c]	172.96	59.22	132.64	129.78[d]	127.36[d]	130.38
$(Phe)_n$	173.32	56.42	37.97	133.45	129.30[e]	128.49

[a] Given as ppm relative to Me_4Si. [b] Poly(L-norvaline). [c] Poly(D-phenylglycine). [d] Signal represents two carbons. [e] Signal represents four carbons.

Table V
Substituent Effects[a] on CO and α-Carbon in Dissolved and Solid Polypeptides

relative position	substituent effects				
	dissolved PP's		solid PP's[b]		alkanes[c]
	CO	α-C	CO	α-C	CH, CH_2, CH_3
α-CH_3		+8.2[d] +8.2[e]		+5.0	+9.1
α-phenyl		+15.1		+11.2	
β-CH_3	+2.5[d] +2.3[e]	+5.7[d] +5.4[e]	+3.0	+4.5[d] +4.5[e]	+9.4
β-phenyl	−0.7	+5.0	0.0	+4.0	
γ-CH_3	−0.5[d] −0.6[e]	−1.4[d] −1.5[e]	−0.2[d] −0.7[e]	−1.2[d] −1.3[e]	−2.5
γ-phenyl	−0.3		+3.2		
δ-CH_3	+0.16[d] +0.13[e]				+0.3

[a] δ given as ppm relative to polyglycine; downfield shifts relative to polyglycine have a positive sign. [b] Pleated sheet structure. [c] n-Alkanes measured in $CDCl_3$ (from ref 36). [d] First substituent. [e] Second substituent.

both chiral centers and H-bonds, its secondary structure is difficult to predict and the ^{13}C NMR spectrum does not allow any decision in this particular case.

Substituent Effects. Paul and Grant concluded from ^{13}C NMR measurements of alkanes in nonpolar solvents that additive substituent effects exist when saturated carbons in α-, β-, γ-, and δ-positions relative to the carbon under investigation are considered[36] (Table IV). We have compared the Paul and Grant shift increments with those found for polypeptides in acidic solution and in the solid state (Table V). A mixture of trifluoroacetic acid and 5 vol % methanesulfonic acid (MSA) was used as solvent because this mixture is the least acidic and least viscous solvent in which all polypeptides are soluble. In the case of solid polypeptides only the β-sheet structures were considered because polyglycine, polyvaline, and poly(phenylglycine) are not available in the helix conformation. For the α-C one finds that saturated carbons in α-positions (e.g., the CH_3 groups) cause a substituent effect of +8.2 ppm (downfield shift relative to Gly) when the polypeptides are measured in solution. The comparison of $(Ala)_2$ and $(\alpha\text{-Aib})_n$ demonstrate the good additivity of this α-increment. This shift effect is in much better agreement with the α-increment of alkanes than with the 5.0-ppm increment observed for solid polypeptides. In contrast, the shift increments of saturated carbons in β-position of dissolved and solid peptides agree better with one another than with the β-increment of alkanes. Furthermore, a comparison of $(Ala)_n$ with $(\alpha\text{-Abu})_n$ and $(Val)_n$ demonstrates a good additivity of the β-effect. This is also true for the γ-effect of CH_3 groups, and the γ-effects of dissolved and solid polypeptides agree better with one another than with that of alkanes. When the peptide carbonyls of dissolved and solid polypeptides are considered, a positive shift increment is observable for β- and δ-substituents and a negative one for γ-carbons. However, as expected for unsaturated carbons, no quantitative relationships with the increments of alkanes exist. Thus, it is obvious that the substituent effect in alkanes and polypeptides exhibits a perfect agreement only with respect to sign but not with respect to magnitude. However, the spectra of polypeptides in solution are useful for signal assignments of solid-state spectra, if shift differences >2 ppm are discussed.

Conclusion

Our ^{13}C NMR CP/MAS measurements of various solid polypeptides demonstrate that this relatively new technique allows one to differentiate between various secondary structures. Compared to X-ray diffraction and IR spectroscopy a quantification of signal intensities seems to be more feasible provided that appropriate acquisition parameters were used. ^{13}C NMR CP/MAS spectra (75.5 MHz) allow one to detect as little as 5% α-helix in the presence of pleated sheet structure or 10% PPII (3_1 helix) in the presence of PPI or vice versa. The quantification allows one to investigate numerous relationships between the reaction conditions of peptide syntheses, on the one hand, and the secondary structures of the reaction products, on the other hand. Furthermore, relationships between secondary structure and various properties of synthetic oligo- and polypeptides may be better understandable. Thus, we believe that the application of high-resolution CP/MAS spectra will considerably improve the characterization of solid peptides.

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Hydrocarbon-Soluble Sulfonating Reagents. Sulfonation of Aromatic Polymers in Hydrocarbon Solution Using Soluble Acyl Sulfates

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ABSTRACT: Previously, we reported that some types of olefin polymers and their small-molecule models were unexpectedly resistant to sulfonation in hydrocarbon solvents. This substantial reactivity difference between chlorinated and hydrocarbon solvents was attributed to the solubility characteristics of the sulfonating agent. Aromatic polymers such as polystyrene are inherently less reactive than olefin polymers and are even more resistant to sulfonation in hydrocarbon solution. We postulated that hydrocarbon-soluble acyl sulfates, unlike acetyl sulfate, would be efficient and rapid reagents for the sulfonation of polymers that are unreactive, and this has been substantiated. Even small improvements in hydrocarbon solubility were found to be beneficial, and completely homogeneous sulfonation was possible with longer chain acyl sulfates. As a result, polystyrene was sulfonated effectively in cyclohexane solution. Long-chain phosphate ester–sulfur trioxide complexes were also soluble in cyclohexane and are suitable reagents for completely homogeneous sulfonation.

Introduction

Homogeneous sulfonation offers some potential utility of both process control and product uniformity. Small-molecule sulfonations sometimes utilize sulfuric acid, oleum, or liquid SO_2 as a mutual solvent for the substrate and sulfonating reagent, but this is not generally convenient with hydrocarbon polymers.

Polystyrene has been sulfonated homogeneously in chlorinated solvents such as dichloroethane by using triethyl phosphate–sulfur trioxide complexes.[2-4] Recently, sulfonation of polystyrene in cyclohexane utilizing H_2SO_4 catalyzed by Ag_2SO_4 has been proposed[5] as an improvement over heterogeneous sulfonation using 100% sulfuric acid. However, there are no good procedures for totally homogeneous sulfonation of polystyrene in a hydrocarbon such as cyclohexane.

We have been studying the sulfonation chemistry of some small-molecule models of EPDM rubbers using acetyl sulfate as the reagent.[6] In the course of these studies we observed that less reactive EPDM rubbers or their small-molecule models were not reactive in hydrocarbon solution using acetyl sulfate reagent. The extremely low reactivity of EPDM rubbers containing dicyclopentadiene or 1,4-hexadiene as the termonomer is in marked contrast to ethylidenenorbornene-containing EPDM, which sulfonates rapidly in hydrocarbon solution. These observations were rationalized by means of model studies which showed that many different olefin types are sulfonated rapidly in methylene chloride in contrast to hexane or pentane solution, wherein only the most reactive olefins are sulfonated. The solvent effect was traced to the low solubility of acetyl sulfate in hydrocarbon solution. Only olefins with very high specific reaction rates could be sulfonated effectively in hydrocarbon solvent where the reagent was in very low concentration; however, all of the different olefin types were extremely reactive under similar conditions using CH_2Cl_2 solvent where the reaction was homogeneous.

Recent interest in lightly sulfonated polystyrene ionomers prompted us to consider the possibility of a hydrocarbon solution process. The effect of homogeneous sulfonation demonstrated with olefin polymers should be even more significant with less reactive aromatic polymers. Indeed, Makowski[7] found that the reaction of acetyl sulfate with polystyrene, which was facile in dichloroethane, was

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