with results of photochemical reaction rates measured by IR spectrometry.

Usually, it may be assumed that the rate of photochemical loss of C=C in a cinnamate polymer, as measured by IR spectrometry, represents the sum of both intra- and intermolecular photochemical reactions of functionalized polymer chains. On the other hand, the practical photosensitivities of coPCNPMA and coPCNNMA measured by the gray-scale method depend only on the rate of interchain cross-linking.

Therefore it can be concluded that though the apparent rate of photochemically induced consumption of polymer-bond cinnamate is strongly decreased by the introduction of large excess amounts of bulky pendant 4-nitro-1-naphthoxy groups, the intermolecular photochemical reactions of the cinnamic ester moieties in coPCNNMA are much more favored than in coPCNPMA, probably because of a biased conformation of the main chain and pendant cinnamic ester moieties in the former polymer due to greater steric hindrance of its more bulky photosensitizing groups.

As a consequence, we propose here that an appropriate conformation of the polymer chain and the photosensitive group is another important factor to consider in designing highly sensitive polymeric negative-working photoresists, along with high molecular weight,[21] high content of photosensitive moiety,[21] and low T_g.[22]

Registry No. coPGMA, 26141-88-8; TEA, 121-44-8; TMAB, 64-20-0; TEAB, 71-91-0; TPAB, 1941-30-6; TBAB, 1643-19-2; TBAI, 311-28-4; TBAP, 1923-70-2; TBPB, 3115-68-2; benzoic acid, 65-85-0.

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Secondary Structure of Peptides. 20. Primary–Secondary Structure Relationships of Ala/Gly/Val Copolypeptides†

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ABSTRACT: Benzylamine-, triethylamine-, and pyridine-initiated terpolymerizations of alanine *N*-carboxyanhydride (Ala-NCA), glycine *N*-carboxyanhydride (Gly-NCA), and valine *N*-carboxyanhydride (Val-NCA) were conducted in various solvents. The molar compositions of the resulting terpolypeptides were determined from ^{1}H NMR spectra. Their sequences were analyzed by means of ^{15}N NMR spectroscopy, and nearly random sequences were found in all cases. The secondary structure of the solid "as-polymerized" samples was characterized by means of ^{13}C NMR CP/MAS spectroscopy. The α-helix/β-sheet ratio was determined for all three amino acid units separately. In contrast to glycine a substantial fraction (up to 60%) of Val units assumes the α-helical conformation. The composition of the secondary structure partially changes upon reprecipitation. In contrast to the random terpolypeptides the two isomeric sequence polypeptides $(Ala\text{-}Gly\text{-}Val)_n$ and $(Gly\text{-}Ala\text{-}Val)_n$ exclusively possess the β-sheet structure. However, in the block copolypeptide $(Ala)_l$-$(Gly)_m$-$(Val)_n$ the $(Ala)_l$ blocks assume the α-helical conformation, whereas the $(Gly)_m$ and $(Val)_n$ blocks exclusively take on the β-sheet structure.

Introduction

Copolypeptides are in principle better models of proteins than homopolypeptides, because their primary structure includes two or more different amino acids. However, their role as protein models suffered in the past from the fact that no routine method of sequence analysis existed, because the procedures used for proteins cannot be applied to copolypeptides prepared by ring-opening polymerization. Furthermore, IR and spectroscopy and X-ray diffraction allowed at best the determination of the most

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abundant secondary structure of the solid samples. In previous papers[1-3] we have demonstrated that ^{15}N NMR spectroscopy of dissolved copolypeptides may enable a satisfactory characterization of their sequences. ^{1}H NMR spectra allow a determination of the molar compositions, and still more important ^{13}C NMR CP/MAS spectroscopy allows one to quantify the composition of the secondary structure of solid polypeptides.[4-7] In favorable cases, such as Ala/Val or Leu/Val copolypeptides it was even feasible to determine the α-helix/β-sheet ratio of each comonomer separately.[8,9]

Thus, it was the purpose of the present work to explore the potential of these new analytical tools by means of ternary polypeptides (terpolypeptides). Terpolypeptides of Ala, Gly, and Val were chosen for two reasons. First, spectroscopic experience with Ala/Gly and Ala/Val copolypeptides was available.[8,10] Second, Ala, Gly, and Val belong to the five or six most abundant constituents of scleroproteins, such as elastin, wool keratins, feather keratins, silk fibroin, etc. Since the peptide chains in such scleroproteins are not or not fully solvated, solid copolypeptides might be better models than dissolved ones.

Experimental Section

Solvents. Dioxane and tetrahydrofuran were twice refluxed and distilled over sodium. Acetonitrile and dimethylformamide were distilled over phosphorus pentoxide. Pyridine and triethylamine were refluxed and distilled over freshly powdered calcium hydride.

Monomers. Gly-NCA was prepared from *N*-(benzyloxycarbonyl)glycine trimethylsilyl ester as described previously.[11] Ala-NCA and Val-NCA were prepared by phosgenation of the corresponding amino acids in a mixture of dioxane and methylene chloride (1:1 by volume). All NCAs were purified with dry charcoal. Ala-NCA and Gly-NCA were twice recrystallized from tetrahydrofuran/chloroform. Val-NCA was twice recrystallized from ethyl acetate/ligroin. After this purification chloride ions were absent as indicated by the $AgNO_3$ test.

Terpolymerizations. A mixture consisting of 40 mmol of Ala-NCA, 20 mmol of Gly-NCA, and 20 mmol of Val-NCA was dissolved in 100 mL of a dry solvent, and the initiator (1 mL) was added in the form of a 1 M solution in dioxane. In the case of no. 6 the monomers were dissolved in 1.2 mol of dry pyridine. Finally, the reaction mixture was poured into 1 L of cold diethyl ether. The precipitated terpolypeptides were isolated by filtration and dried at 60 °C in vacuo.

Block Copolymerization. Ala-NCA (30 mmol) was polymerized in 50 mL of dry dioxane by means of 0.6 mL of a 1 M solution of benzylamine. After 2 days the poly(alanine) was precipitated from 500 mL of cold, dry diethyl ether, isolated by filtration, and added (without drying) to a solution of 30 mmol of Gly-NCA in 80 mL of dry dioxane. After 2 days a solution of 30 mmol of Val-NCA in 20 mL of dry dioxane was added to the reaction mixture and homogenized by vigorous shaking. The reaction vessel (closed with a freshly prepared calcium chloride drying tube) was stored at ca. 25 °C for 7 days. Afterward, the reaction mixture was worked up as described above.

Sequence Polypeptides. The free oligopeptides H-Ala-Val-Gly-OH and H-Val-Ala-Gly-OH were prepared in a stepwise manner from Boc-Ala-OH or Boc-Val-OH and amino acid methyl esters by means of 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ).[12] The methyl ester groups were saponified by means of 1 N NaOH in a water/ethanol mixture, and the Boc group was finally removed by means of TFA at room temperature. The free tripeptides were purified by reprecipitation from acetic acid/2-propanol and characterized by means of C, H, and N elemental analyses. Portions (50 mmol) of the tripeptides were condensed in 50 mL of dry dimethylformamide for 2 days at 80 °C using (diethoxyphosphino)-1,3,4-triazole as condensing reagent.[13] The sequence polypeptides were precipitated from ca. 300 mL of water, and they were reprecipitated from formic acid/methanol, refluxed with 2 N ammonia, and reprecipitated again from formic acid/methanol. Even this extensive purification did not remove contamination with phosphorus derivatives completely as indicated by elemental analyses. $(Ala\text{-}Val\text{-}Gly)_n$: yield 27%. Anal. Calcd for $C_{10}H_{17}N_3O_3$ (227.27): C, 52.85; H, 7.54; N, 18.49. Found: C, 52.01; H, 7.03; N, 17.89. $(Val\text{-}Ala\text{-}Gly)_n$: yield 31%. Found: C, 51.98; H, 7.11; N, 17.81. The slight contamination with phosphorus derivatives did not affect the usefulness of the sequence polypeptides as spectroscopic models (Figure 4).

^{1}H NMR Measurements. The 360-MHz ^{1}H NMR spectra were obtained on a Bruker AM 360 FT spectrometer in 5-mm-o.d. sample tubes. Polypeptide (50 mg) was dissolved in 1 mL of deuterated TFA containing Me_4Si as internal standard and was measured immediately after dissolution, because the C_α protons undergo slow H/D exchange.[14] The following chemical shifts were observed: δ 1.05 (s), 1.56 (s), 2.18 (s), 4.32 (s), 4.49 (s), 4.72 (s).

^{15}N NMR Measurements. The 40.5-MHz ^{15}N NMR spectra were obtained on a Bruker AM-400 FT spectrometer in 15-mm-o.d. sample tubes with a 5-mm-o.d. coaxial tube containing a solution of $^{15}NH_4{}^{15}NO_3$ in D_2O (the NO_3^- ion served for shift referencing). Solutions of 600 mg of polypeptide in 4.5 mL of TFA were measured by means of the INEPT pulse sequence[15] optimized for $^{1}J_{NH} = 92$ Hz. The INEPT pulse sequence gave ca. 6 times better signal to-noise ratio than inverse-gated decoupling. Ca. 9000–12000 transients were accumulated. The following chemical shifts (ppm relative to NO_3^-) were found for the terpolypeptides: −266.7 (Ala-Gly); −265.8 (Gly-Gly); −262.9 (Val-Gly); −254.3 (Ala-Val); −253.5 (Gly-Val); −250.4 (Ala-Ala); −250.1 (Gly-Ala); −248.8 (Val-Val); −246.6 (Val-Ala).

^{13}C NMR CP/MAS Measurements. The 75.4-MHz ^{13}C NMR CP/MAS measurements were obtained on a Bruker CXP-300 FT spectrometer. Samples of ca. 100 mg were measured in a "double-bearing rotor" made of boron nitride. A spinning rate of ca. 4000 Hz was applied along with a single-pulse sequence with a contact time of 1 ms and a pulse repetition time of 4 s. Depending on the density of the sample, between 400 and 600 transients were accumulated. The chemical shifts listed in Table I were referenced to Me_4Si as described previously.[16]

^{13}C NMR Solution Measurements. The 90.5-MHz ^{13}C NMR measurements were obtained on a Bruker AM 360 FT spectrometer. Samples of 300 mg dissolved in 2 mL of TFA were measured in 10-mm-o.d. sample tubes with a coaxial 4-mm-o.d. tube containing a mixture of Me_4Si and dioxane (1:1 by volume). The following acquisition parameters were used: pulse width 6 μs (ca. 45 °C), 1-s relaxation delay, 32 K data points/20 000-Hz spectral width; ca. 11 000 transients in the case of terpolypeptides (Figure 6). The chemical shifts are listed in Table I.

Results and Discussion

Primary Structure. In the present work three different types of Ala polypeptides containing alanine, glycine, and valine were investigated. Copolypeptides with nearly random sequences of the three amino acids, $(Ala\text{-}Gly\text{-}Val)_n$, were prepared by copolymerization of mixtures of alanine *N*-carboxyanhydride (Ala-NCA), Gly-NCA, and Val-NCA at a molar ratio of 2:1:1 (Table I). A three-block copolypeptide was synthesized by batchwise copolymerization of Ala-NCA (first block), Gly-NCA (second block), and Val-NCA (third block) in a molar ratio of 2:1:1. For this block copolypeptide the following abbreviation will be used: $(Ala)_l\text{-}(Gly)_m\text{-}(Val)_n$. Finally two terpolypeptides with regular retroisomeric[17] sequences, namely $(Ala\text{-}Gly\text{-}Val)_n$ and $(Gly\text{-}Ala\text{-}Val)_n$, were obtained by condensation of the tripeptides H-Val-Ala-Gly-OH and H-Ala-Val-Gly-OH by means of (diethoxyphosphino)-1,3,4-triazole.[18]

Since secondary structure of polypeptides and proteins is believed to depend mainly on their primary structure, it was the first aim of this work to characterize the primary structure of the copolypeptides under investigation. In the case of synthetic copolypeptides the primary structure is defined by the following five parameters: (A) average molecular weight ($\bar{M}_n$ or $\bar{M}_w$) or average degree of polymerization ($\overline{DP}$), (B) molecular weight distribution (MWD), (C) molar ratio of comonomers, (D) sequence of the comonomers, and (E) nature of the end groups.

Table I
^{13}C NMR Chemical Shifts of Ala-, Gly-, and Val-Containing Polypeptides Measured in TFA Solution or in the Solid State[a]

polypeptide	NMR method and confirmation	δ of Ala			δ of Gly		δ of Val			
		CO	C_α	C_β	CO	C_α	CO	C_α	C_β	C_γ
(L-Ala)$_n$	CP/MAS, αh	176.6	52.8	15.5						
	βs	172.2	49.2	20.1						
	TFA, αh	176.4	51.3	15.7						
(D,L-Ala)$_n$	TFA, coil	175.1	50.8	16.2						
(Ala-Gly-Val) random terpolypeptide	CP/MAS, αh	176.0	52.8	15.6	172.2	44.3	175.9	64.6	29.0	18.9
	βs	172.3	49.1	18.9	169.1	43.3	172.3	58.3	32.9	
	TFA, coil	176.3	50.7	16.1	172.9	43.2	173.9	66.5	31.1	17.8
		175.3			172.0					17.3
	TFA/MAS,[b] coil	176.5	51.3	16.1	173.4	43.3	174.5	66.5	31.1	17.8
		175.9			172.7					17.3
(Ala-Gly-Val)$_n$	CP/MAS, βs	172.7	48.6	18.7	168.3	43.1	170.0	56.8	34.0	18.7
	TFA, coil	175.9	50.6	16.9	172.2	43.1	173.8	60.5	31.0	17.9
										16.9
(Gly-Ala-Val)$_n$	CP/MAS, βs	171.8	48.8	18.9	168.8	43.0	171.8	57.3	33.8	18.9
	TFA, coil	175.6	50.8	16.3	171.8	43.1	174.8	60.8	31.1	17.8
										17.0

[a] Margin of error ±0.2 ppm. [b] TFA containing 5 vol % methanesulfonic acid.

Table II
Reaction Conditions and Results of Various Terpolymerizations of Gly-NCA, L-Ala-NCA, and L-Val-NCA (Mole Ratio 1:2:1)

no.	initiator (monomer/initiator mole ratio)	solvent	temp, °C	time, h	yield,[a] %	mole ratio[b] (Ala-Gly-Val)$_n$	$\overline{DP}$[c]
1	benzylamine (80/1)	dioxane	20	8	98	1.00:0.41:0.40	80
2	benzylamine (80/1)	acetonitrile	20	8	67	1.00:0.47:0.19[d]	59
3	benzylamine (80/1)	dimethylformamide	20	8	76	1.00:0.47:0.24	65
4	triethylamine (80/1)	dioxane	20	8	88	1.00:0.42:0.37	
5	triethylamine (80/1)	acetonitrile	20	8	20		
6	pyridine (1/15)	pyridine	20	8	67	1.00:0.45:0.19	

[a] Referred to the theoretical molar sum of all three monomer units. [b] Determined from ^1H NMR spectra. [c] Determined by ^1H NMR end-group analyses; margin of error ±4. [d] Hydrolytic (6 N HCl) amino acid analysis gives 1.00:0.55:0.14.

Since end groups certainly do not influence the secondary structure of high molecular weight polypeptides, four parameters need to be discussed. The insolubility of Ala-, Gly-, and Val-containing copolypeptides in common organic solvents prevents the application of all physicochemical methods normally used for the determination of $\bar{M}_n$'s, $\bar{M}_w$'s, and MWDs. Therefore, the block copolypeptide and three random copolypeptides (No. 1–3, Table II) were prepared by benzylamine-initiated polymerizations and subjected to ^1H NMR spectroscopic end-group analyses. Primary amine initiated polymerizations of α-amino acid NCAs are known to follow the pattern of "living polymerizations", at least for monomer/initiator ratios below 100. Thus, each peptide chain must contain one benzylamide end group, and the aromatic protons of the benzyl group, which give a singlet ^1H NMR signal, are easy to measure in an aliphatic polypeptide as demonstrated previously.[5,6]

These ^1H NMR spectroscopic end-group analyses confirmed that the corresponding polymers obey eq 1, in

$$\overline{DP} = \frac{M}{I}\,\frac{\text{conversion}}{100} \qquad (1)$$

agreement with the living character of the benzylamine-initiated polymerizations. Some crude information on the $\overline{DP}$'s of the other copolypeptides was obtained by comparison of their viscosities with those of no. 1–3 of Table II.

Exact measurements of the MWD were not feasible, because the commercially available GPC apparatus does not work reliably when TFA or stronger acids are required as solvents. However, it is well-known from various homopolypeptides prepared from α-amino acid NCAs that they possess a bimodal MWD. The first weak maximum in these MWDs results from a small fraction of oligomers (DP < 10) that precipitate in an early stage of the polymerization from the reaction mixture due to association via H bonds. Because the copolypeptides prepared in this work are also insoluble in their reaction mixtures and precipitated soon after addition of the initiator, it is highly probable that they contain a small fraction (≤5%) of oligomers that assume the β-sheet structure regardless of their composition. However, because the $\overline{DP}$'s of all samples in this work were >50, the fraction of oligomeric β-sheet should never exceed 5% and, thus, does not affect the characterization of the secondary structure described below.

A parameter with great influence on the secondary structure of copolypeptides is, of course, their molar composition. As demonstrated previously Val-NCA is less reactive than Ala-NCA and in particular Gly-NCA. In the present work the molar compositions, determined from ^1H NMR spectra, show that Val-NCA was indeed never completely incorporated into the copolypeptides, regardless of whether simultaneous or batchwise copolymerizations were conducted (Table II). The most complete conversions of Val-NCA (ca. 70–80%) were found for no. 1 and 4 of Table II and for the block copolypeptide.

The last but important parameter of the primary structure is the sequence of the comonomers. As demonstrated previously,[1-3] ^{15}N NMR spectroscopy is best suited to characterize the sequences of synthetic copolypeptides. The spectra of the (Ala-Gly-Val)$_n$ copolypeptides indicate that an almost perfectly random copolymerization of Ala-NCA and Gly-NCA occurred under all conditions listed in Table II. However, in the case no. 1 and 4 the Val units form slightly longer blocks than expected on the basis of their molar ratio (Figure 1A). This finding agrees

Table III
Sequences and Secondary Structures of (Ala-Gly-Val)$_n$

	av block length[a]			αh/βs					
				"as-polymerized" samples[b]			reprecipitated samples[b]		
no. of Table I	$\bar{L}_A$	$\bar{L}_G$	$\bar{L}_V$	Ala	Gly	Val	Ala	Gly	Val
1	2.70	1.50	1.60	45:55	20:80	60:40	45:55	20:80	33:67
2	2.20	1.60	1.00	25:75	20:80	40:60	40:60	20:80	55:45
3	2.35	1.70	1.25	10:90	10:90	15:85	30:70	10:90	30:70
4	3.20	1.40	1.65	50:50	20:80	60:40	60:40	20:80	25:75
5	—	—	—	—	—	—	—	—	—
6	2.80	1.70	1.20	20:80	10:90	10:90	30:70	10:90	35:65

[a] Determined from ^{15}N NMR spectra; margin of error ±0.1 [b] Determined from ^{13}C NMR CP/MAS spectra; margin of error ±5%.

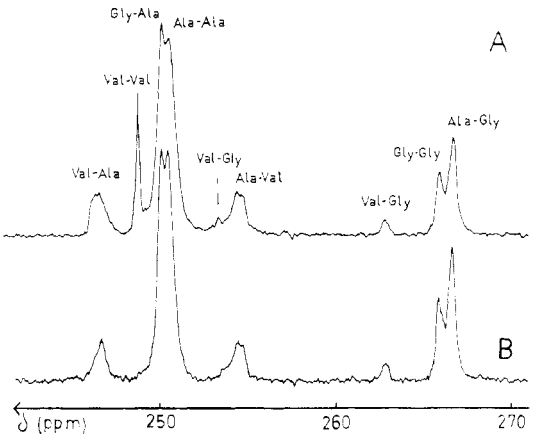

Figure 1. 40.5-MHz ^{15}N NMR INEPT spectra of (Ala-Gly-Val) copolypeptides measured in TFA: (A) sample no. 1, Tables II and III; (B) sample no. 2, Tables II and III.

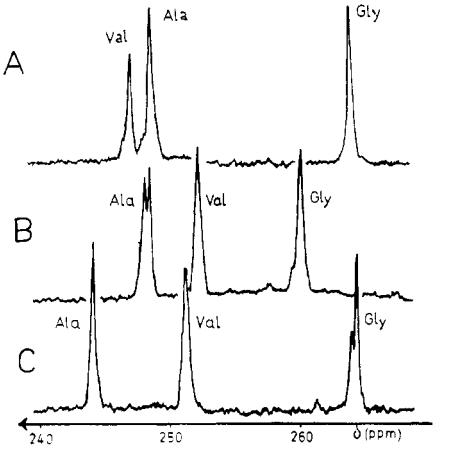

Figure 2. 20.3-MHz ^{15}N NMR inverse-gated-decoupling spectra measured in TFA + 5% methanesulfonic acid: (A) block copolypeptide (Ala)$_l$-(Gly)$_m$-(Val)$_n$; (B) sequence polypeptide (Gly-Ala-Val)$_x$; (C) sequence polypeptide (Ala-Gly-Val)$_x$.

with previous results showing that part of Val-NCA is left for homopolymerization, when Ala-NCA and Gly-NCA were already completely consumed. The average block lengths of the random copolypeptides were calculated according to eq 2, where $\bar{L}_A$, $\bar{L}_G$, and $\bar{L}_V$ are average block

$$\bar{L}_A = I_{AA}/(I_{AG} + I_{AV}) + 1 \quad (2a)$$

$$\bar{L}_G = I_{GG}/(I_{GA} + I_{GV}) + 1 \quad (2b)$$

$$\bar{L}_V = I_{VV}/(I_{VA} + I_{VG}) + 1 \quad (2c)$$

lengths of Ala, Gly, and Val units and I_{AA}, I_{AG}, I_{AV}, etc. are signal intensities of Ala-Ala, Ala-Gly, Ala-Val bonds, etc. The ^{15}N NMR spectra also prove that the batchwise copolymerization led to a three-block copolymer (Figure 2A). Furthermore, they confirm that (Ala-Gly-Val)$_n$ and (Gly-Ala-Val)$_n$ possess regular, nonidentical sequences that were not modified by transpeptidation processes during the condensation at 80 °C (Figure 2B,C). However, as known from various polycondensations of activated oligopeptides,[19] the two sequence polypeptides studied in this work seem to be partially racemized. The splitting of ^{15}N NMR signals in Figure 2B,C along with relatively low optical rotation values (compared to those for the random copolypeptides) point in this direction.

Secondary Structure. As shown previously[8] the ^{13}C NMR signals of C_α and C_β of both Ala and Val units are so well separated that the α-helix and β-sheet peaks of each amino acid are separately observable. Hence, the α-helical and β-sheet fraction of each amino acid can be separately determined from the peak intensities (Figure 3). In the case of Gly units the C_α signal is not sensitive to conformational changes. However, the CO signal splits into an α-helix and a β-sheet peak with a shift difference (Δδ) of ca. 3.5 ppm. The β-sheet peak, which shows up at ca. 168.5 ppm, is detectable in the presence of other amino acids, whereas the α-helix peak of Gly overlaps with the β-sheet

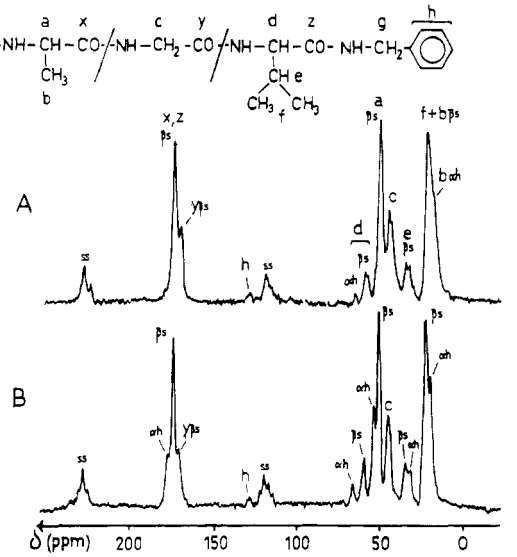

Figure 3. 75.3-MHz ^{13}C NMR CP/MAS spectra of solid (Ala-Gly-Val)$_x$ copolypeptide no. 3, Tables II and III: (A) "as polymerized"; (B) after reprecipitation.

peaks of Ala and Val (Figures 3 and 4). Thus only a crude estimation of α-helix/β-sheet ratio (αh/βs) of the Gly units is feasible. Whether the acquisition parameters used for the cross polarization/magic angle spinning measurements are suited for the quantification of signal intensities was checked with Ala/Val[8] and Ala/Gly[20] copolypeptides.

When the secondary structures of the (Ala-Gly-Val)$_n$ terpolypeptides are compared, it is conspicuous that the αh/βs ratio of the Ala units of the "as-polymerized" samples varies from 10:90 to 50:50, whereas that of the Gly units is nearly constant (Table III). Because the Ala/Gly

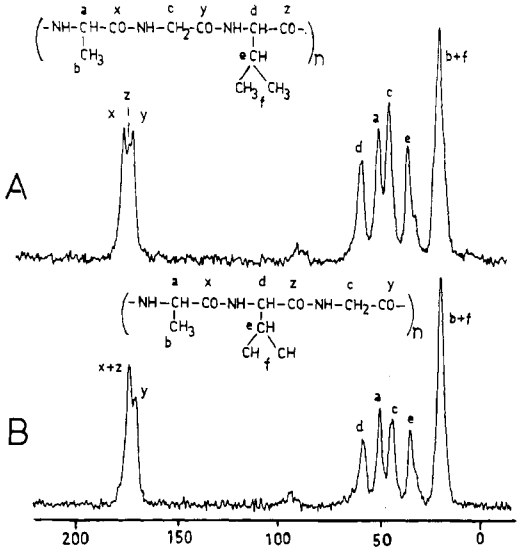

Figure 4. 75.3-MHz ^{13}C NMR CP/MAS spectra of the two solid, retroisomeric sequence polypeptides.

ratio of all samples of Tables II and III is nearly constant and because all sequences are close to randomness, such a broad variation of the $\alpha h/\beta s$ ratio is amazing. However, reprecipitation from TFA/water revealed that the composition of the secondary structure of the "as-polymerized" samples does not represent a thermodynamically stable state, i.e., an equilibrium situation controlled by the primary structure. The $\alpha h/\beta s$ ratios of the Ala units are higher after reprecipitation and display smaller differences when the five samples are compared with each other (Table III). The role of the sequence is evident from the finding that the highest $\alpha h/\beta s$ ratio of Ala units correlates with the greatest average block length (no. 4). The lowest $\alpha h/\beta s$ ratio is shown by a sample with a relatively low average block length (no. 3). Nonetheless, an exact parallel between block lengths and α-helix content is not expected, because the Ala/Val ratio of the five samples is not constant and because no information on the chemical heterogeneity of first and second order is available.

When the $\alpha h/\beta s$ ratio of the Val units is considered, the high α-helix content of the Val units is noteworthy. It is particularly conspicuous that the highest $\alpha h/\beta s$ ratio is found for samples with relatively large average block lengths (no. 1 and 4), i.e., samples where a high β-sheet content is expected for the Val units. Also in these two cases reprecipitation provides an explanation, because the $\alpha h/\beta s$ ratios considerably decrease. Obviously polymerization in the least polar solvent (i.e., dioxane) forces the Val units into the compact α-helical structure, even if the primary structure does not favor this conformation. On the other hand, it is also noteworthy that the $\alpha h/\beta s$ ratios of the Val units in no. 2, 3, and 6 increase upon reprecipitation. This means that depending on the reaction conditions, both thermodynamically unstable α-helices and β-sheet are formed. This result agrees well with that obtained from Ala/Val copolypeptides.[8]

The influence of the sequence on the secondary structure of Ala-, Gly-, and Val-containing copolypeptides is still more evident when the two sequence polypeptides $(Ala\text{-}Gly\text{-}Val)_n$ and $(Gly\text{-}Ala\text{-}Val)_n$ along with the block copolypeptide $(Ala)_l\text{-}(Gly)_m\text{-}(Val)_n$ are taken into consideration. The ^{13}C NMR CP/MAS spectra of the two sequence polypeptides (Figure 4) display, as expected, signals that all agree with a β-sheet structure. Of course, it might be objected that the ratio of helicogenic and nonhelicogenic amino acids is 1:2 in the sequence polypeptides, but $\geq$1:1

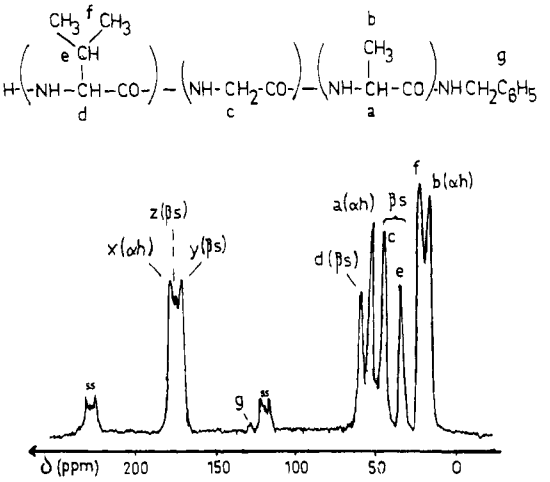

Figure 5. 75.3-MHz ^{13}C NMR CP/MAS spectrum of the solid three-block copolypeptide $Ala)_l\text{-}(Gly)_m\text{-}(Val)_n$ "as polymerized".

in the terpolypeptides. However, it is known from sequence polypeptides such as $(Ala\text{-}Gly)_n$[21] and (Ala-Ala-Gly)[22] that the β-sheet structure is the most stable conformation despite Ala/Gly ratios $\geq$1. Because both sequence polypeptides possess the β-sheet structure it is interesting to see that the pattern of the CO signals is not identical. Obviously, the retroisomerism of both sequences causes neighboring residue effects, which are independent of the main-chain conformation. This conclusion is supported by solution measurements which indicate a similar neighboring residue effect (Table I).

The ^{13}C NMR CP/MAS spectrum of the block copolypeptide exhibits the expected block character of the secondary structure (Figure 5). The Gly and Val blocks exclusively adopt the β-sheet structure, whereas the Ala units mainly assume the α-helical conformation. The small fraction of Ala units in the β-sheet structure obviously originates from the fraction of oligomers ($\overline{DP} \leq 10$) precipitated in the first stage of the polymerization. From the homopolymerization of Ala-NCA it is known[5] that this oligomeric fraction amounts to ca. 10% when $\overline{DP}$ reaches 50. In agreement with this interpretation reprecipitation does not change the secondary structure.

Finally, it is interesting to compare the helix stability of (Ala) blocks in the solid state and in solution. If ca. 5% of the Ala units in terpolypeptides having a β-sheet structure belong to oligomers, the data of Table II indicate that an average block length around 3 ($\pm$0.3) suffices to bring about 50–65% α-helix content. Taking into account the β-sheet structure of $(Ala\text{-}Ala\text{-}Gly)_n$,[22] it may be concluded that sequences of Ala units may accommodate up to 20 mol % Gly or Val units before the α-helix becomes unstable. However, when (Ala-Gly) or (Ala-Val) copolypeptides are measured in TFA solution under equilibrium conditions[23] even 2 mol % Gly or 5 mol % Val units brings about a considerable decrease of the α-helix/random coil ratio. Hence, complete α-helix $\rightarrow$ random coil transition is expected when the terpolypeptides of Tables II and III are dissolved in TFA. The ^{13}C NMR measurements displayed in Figure 6 and Table I clearly confirm this expectation. In other words, solution measurements under equilibrium conditions do not allow quantitative or semiquantitative predictions on the secondary structure of solid copolypeptides and proteins. This aspect is important for the understanding of the solid or poorly solvated polypeptides in membranes or scleroproteins, because the only set of quantitative data on the helicogeneity of amino

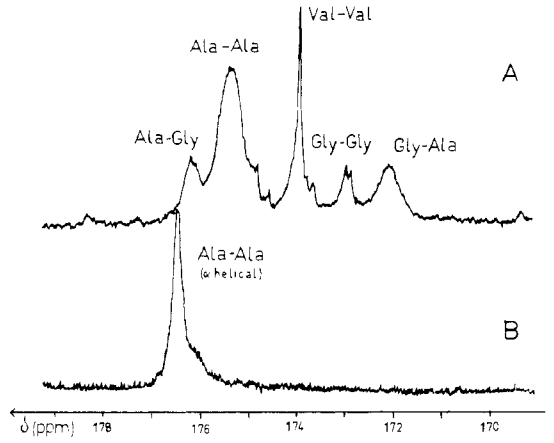

Figure 6. 90.5-MHz ^{13}C NMR spectra of (A) $(Ala\text{-}Gly\text{-}Val)_n$ no. 4 (Table II) in TFA and (B) α-helical $(Ala)_n$, $\overline{DP}$ = 100, in TFA.

acids was derived from solution measurements.[24]

Conclusions

The present investigation of Ala-, Gly-, and Val-containing ternary polypeptides revealed that a combination of ^1H, ^{15}N, and ^{13}C NMR spectroscopy enables at least a semiquantitative description of their primary and secondary structure. It may be concluded that in the solid state regular secondary structures are largely favored over amorophous (or random coil) structures, even when random sequences of helicogenic and nonhelicogenic amino acids are present. Furthermore, the α-helix-breaking influence of nonhelicogenic amino acids may be less effective in the solid state than in solution. Furthermore, the present measurements confirm that "as-polymerized" copolypeptides prepared from NCAs usually possess both thermodynamically unstable β-sheets and α-helices. These thermodynamically unstable conformations can be transformed into stable ones by reprecipitation from cold water or by means of hot water vapor (described in a later part of this series). Hence, such conformational changes might serve as models of denaturation processes such as the heat treatment of hen egg-white proteins. To what extent this analogy is correct is certainly worth future investigation.

Registry No. L-Ala-NCA, 2224-52-4; Gly-NCA, 2185-00-4; L-Val-NCA, 24601-74-9; $(Ala\text{-}Gly\text{-}Val)_n$ (copolymer), 78677-25-5; $(Ala\text{-}Val\text{-}Gly)_n$ (homopolymer), 69288-26-2; $(Ala\text{-}Val\text{-}Gly)_n$ (SRU), 56959-28-5; $(Val\text{-}Ala\text{-}Gly)_n$ (homopolymer), 65503-85-7; $(Val\text{-}Ala\text{-}Gly)_n$ (SRU), 56959-29-6; H-Ala-Val-Gly-OH, 69288-25-1; H-Val-Ala-Gly-OH, 54769-86-7; ^{15}N, 14390-96-6.

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