300 °C, the sample showed no indication of melting.

Figure 4 shows DSC curves of un-cross-linked (0% DCP) and cross-linked (40% and 200% DCP) polyethylene films drawn to $\lambda = 50$ and 100. The profile of the un-cross-linked films shows a large main peak around 148 °C in addition to smaller shoulders. In contrast, the peak position of all cross-linked films, except the film (200% DCP) drawn to $\lambda = 50$, shifts to 145 °C. In addition to the endotherms arising from melting of the crystalline regions, the profiles also show exotherms in the range from 194 to 284 °C. Examination of the specimen after the DSC experiments leads to the conclusion that these exotherms arise from oxidation and decomposition processes. It can be seen from the profiles that these effects occur at higher temperatures for the cross-linked than for un-cross-linked films. The specimen (200% DCP) drawn to $\lambda = 50$ shows three small peaks around 130, 140, and 150 °C. This indicates that considerable cross-linking causes a decrease in crystallinity owing to an increase in cross-linking in the amorphous phase.

In summary, measurements of the complex dynamic moduli have shown that for samples drawn to $\lambda = 50$ and extensively cross-linked (for example 200% DCP), there is a considerable improvement in high-temperature resistance, with maintenance of tensile properties up to 230 °C, but the storage modulus at 230 °C is only about 0.5 GPa. In contrast, samples drawn up to $\lambda = 100$ and cross-linked with a suitable amount of DCP (for example, 40% DCP) show a storage modulus greater than 110 GPa at 20 °C and it is still 2.0 GPa even at 200 °C, but the specimen cannot be maintained beyong 200 °C, because it breaks However, the latter specimen can be certainly termed "high-modulus polyethylene with heat-resistant properties", since un-cross-linked ultra-high-modulus polyethylene cannot exist at temperatures beyond 150 °C.

Acknowledgment. We are indebted to Prof. A. J. Pennings, State University of Groningen, Groningen, The Netherlands, for valuable discussions, comments, and suggestions. We are also grateful to Dr. R. St. John Manley, Department of Chemistry, McGill University, for his kind help with the English presentation.

Registry No. (DCP)(E) (copolymer), 106705-26-4.

References and Notes

(1) Capaccio, G.; Ward, I. M. *Polymer* **1974**, *15*, 233.
(2) Gibson, A. G.; Ward, I. M. *J. Mater. Sci.* **1980**, *15*, 979.
(3) Kohima, S.; Desper, C. R.; Porter, R. S. *J. Polym. Sci., Polym. Phys. Ed.* **1978**, *16*, 1721.
(4) Smook, J.; Torfs, J. C.; van Hulten, P. F.; Pennings, A. J. *Polym. Bull.* **1980**, *2*, 293.
(5) Barham, P. J.; Keller, A. *J. Mater. Sci.* **1980**, *15*, 2229.
(6) Smith, P.; Lemstra, P. J. *Polymer* **1980**, *21*, 1341.
(7) Smith, P.; Lemstra, P. J.; Pippers, J. P. L.; Kiel, A. M. *Colloid Polym. Sci.* **1981**, *25*, 1070.
(8) Sawatari, C.; Matsuo, M. *Colloid Polym. Sci.* **1985**, *263*, 783.
(9) Matsuo, M.; Sawatari, C. *Macromolecules* **1986**, *19*, 2036.
(10) Matsuo, M.; Sawatari, C. *Macromolecules* **1986**, *19*, 2028.
(11) deBoer, J.; van den Berg, H.-J.; Pennings, A. J. *Polymer* **1984**, *25*, 513.
(12) Manabe, S.; Sakoda, A.; Katada, A.; Takayanagi, T. *J. Macromol. Sci. Phys.* **1970**, *B4*, 161.
(13) Suehiro, S.; Yamada, T.; Inagaki, H.; Kyu, T.; Nomura, S.; Kawai, H. *J. Polym. Sci., Polym. Phys. Ed.* **1979**, *17*, 763.

Template Effect on the Copolymerization of L-Alanine NCA and Sarcosine NCA

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ABSTRACT: Copolymerization of L-Ala NCA and Sar NCA catalyzed by poly(2-vinylpyridine) template was carried out. The effect of concentration and degree of polymerization of the template on the sequence of the resulting copolypeptide was determined by ^{13}C NMR. It was found that only the higher of the two molecular weight templates used had an effect on the sequence of the copolypeptide. Molecular weights of the copolypeptides were determined by gel permeation chromatography.

Introduction

Template polymerization, also termed replica or matrix polymerization, was first suggested by Szwarc.[1] In such systems the monomers are complexed or adsorbed on a macromolecular template and polymerized for at least the greater part of their growth. The mechanism of propagation of the growing chain may affect not only the reaction rate but also the molecular weight and the microstructure of the polymer formed. In most cases the presence of a template leads to rate enhancement. This phenomenon has been referred to as "chain effect" by Ballard and Bamford,[2] who first extensively studied this field. Since then many template systems have been investigated in various laboratories around the world,[3] involving free radical vinyl polymerizations.

Imanishi et al.[3a,4] found that poly(2-vinylpyridine) (P(2VPy)) catalyzes the polymerization of D,L-β-phenylalanine *N*-carboxy anhydride (NCA) faster than α-picoline. They showed that P(2VPy) adsorbs the NCA monomer by hydrogen bonding, which increases the local concentration of NCA. Since the polymer chain is flexible, the intramolecular reaction of the "activated" NCA[5] along the chain takes place frequently, resulting in a faster polymerization.

In the present study the effect of P(2VPy) on the copolymerization of L-Ala NCA and Sar NCA was determined. Since only L-Ala NCA is able to hydrogen bond to a P(2VPy) template (see Figure 1) its local concentration along the polymer catalyst should be higher than in solution, resulting in a copolypeptide with a block nature in L-Ala.

Procedure

NCA polymerizations were carried out in a nitrobenzene solution at 25 °C, at a constant monomer concentration of 0.50 M. The polymerization was started by mixing a catalyst solution with

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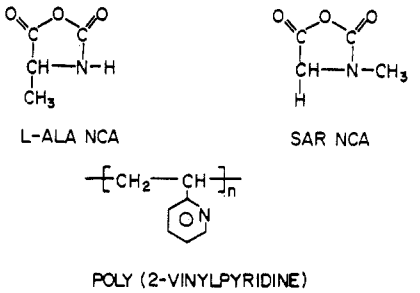

Figure 1. Monomers and template used in this study.

Table I
Composition of Copolypeptides

catalyst	poly-peptides	mol % Ala[a]	mol % Sar[a]	mol % P(2VPy)[b]
2-vinylpyridine	CP-1	0.50	0.50	
low MW P(2VPy)[c]	CP-2	0.52	0.48	0.25
	CP-3	0.50	0.50	0.50
	CP-4	0.73	0.27	0.50
	CP-5	0.22	0.78	0.50
high MW P(2VPy)[d]	CP-6	0.45	0.55	0.25
	CP-7	0.56	0.44	0.50
	CP-8	0.51	0.49	1.00
	CP-9	0.50	0.50	1.50

[a] Mole ratio of monomer units determined by 60-MHz ^{1}H NMR. [b] The P(2VPy) concentration refers to the concentration of 2-vinylpyridine residues in the polymeric template to the total monomer concentration. [c] Molecular weight of 3100, determined by GPC. [d] Molecular weight of 20 000 purchased from Aldrich Chemical Co.

an NCA solution. The catalyst concentration refers to the concentration of 2-vinylpyridine residues in the polymeric template to the total monomer concentration (mol/mol). The polymers were allowed to react to completion and were precipitated with acetone. P(2VPy) was separated from the copolypeptide by extraction with dioxane.

The ^{13}C NMR spectra were obtained by a Varian XL-300 NMR spectrometer at 25 °C, using ca. 200 mg/mL solutions of the copolypeptide in deuterio-trifluoroacetic acid with Me_4Si as internal standard. T_1 values for all carbons were determined, the longest being 1 s. The spectra were acquired with an inverse-gated decoupling sequence using a 5.0-s relaxation delay between scans. This sequence gives decoupled spectra with no heteronuclear NOE. Therefore, the spectra should be quantitative. The composition of the copolypeptides were determined by 60-MHz ^{1}H NMR spectroscopy using the integrated intensities of the methyl group signals.

The apparent molecular weights of the polypeptides were determined by gel permeation chromatography (GPC) at room temperature using hexafluoro-2-propanol as the solvent and are relative to nylon 66. The calibration curve was constructed by the linear molecular weight distribution method.[13] The broad molecular weight standard nylon 66 has the following reported average molecular weights: M_n = 49 000 (VPO) and M_w = 91 500 (ultracentrifugation). All samples were run in hexafluoro-2-propanol/0.01 M sodium trifluoroacetate at 0.3 mL/min using a series of 1-E125 and 1-E1000 Bondagel columns from Waters Associates, Inc., in series with a 1-160 diol-type column packed at Jordi Associates. The 160 column was added to increase low end resolution. The injection size was 100 μL of 0.5% w/v and the samples were monitored at 32X on a Waters Model 401 refractive index detector and simultaneously at 230 nm on a Waters Model 450 UV detector.

Materials. L-Ala NCA and Sar NCA were prepared by the method of Leuchs.[6,7] The monomers were recrystallized repeatedly from ethyl acetate/hexane until the chloride content was negative.[8] Low molecular weight poly(2-vinylpyridine) was synthesized in toluene solution using phenylmagnesium bromide catalyst[9] at 45 °C. The molecular weight was determined to be 3100, relative to polystyrene, by GPC. High molecular weight poly(2-vinylpyridine), molecular weight 20 000, was purchased from Aldrich Chemical Co. Solvents were rigorously purified to remove all traces of water. All manipulations of NCA monomer were done in a glovebox.

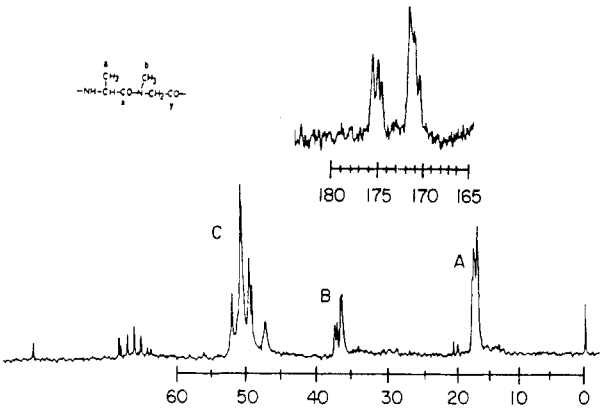

Figure 2. 75.5-MHz ^{13}C NMR of polypeptide CP-1 (TFA-D_1).

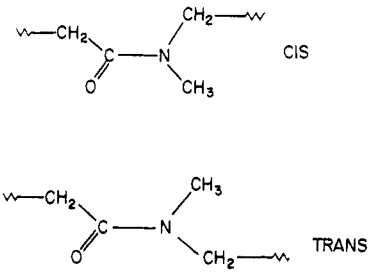

Figure 3. Cis/trans isomerism of the *N*-actyl sarcosyl bond.

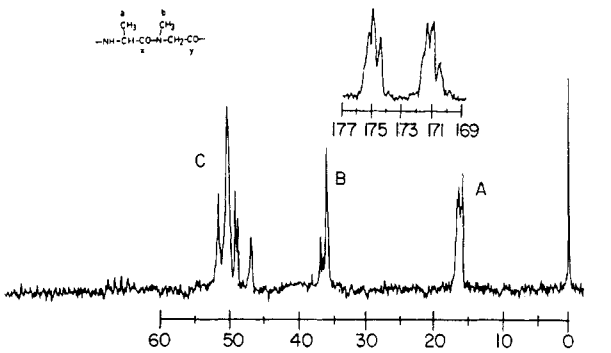

Figure 4. 75.5-MHz ^{13}C NMR spectrum of polypeptide CP-2 (TFA-D_1).

Results and Discussion

Table II summarizes the chemical shifts of the carbon atoms in the polypeptides. Figure 2 shows the ^{13}C NMR spectrum of polypeptide CP-1. The spectra shows that all signals are split due to the cis/trans isomerism of the *N*-acyl sarcosyl bond;[10] see Figure 3. The cis/trans isomerism of the *N*-acyl sarcosyl bond produces 2^n splittings of a given NMR resonance, where n is the number of peptide bonds that can produce an effect observable by NMR. The carbonyl region for L-Ala and Sar show three distinct resonance each, corresponding to a homopeptide linkage and two copeptide linkages (cis/trans) for L-Ala and one copeptide linkage and two homopeptide linkages for Sar.[11] This, along with the partial cis/trans splitting of all the L-Ala signals, clearly indicates that a copolypeptide was obtained and not a mixture of homopolypeptides. The copolypeptide obtained possesses an L-Ala/Sar ratio of 1:1 (see Table I), which indicates that the reactivities of L-Ala NCA and Sar NCA are similar, which leads us to conclude that a random copolypeptide was obtained under the reaction condition of copolymerization catalyzed by 2-vinylpyridine.

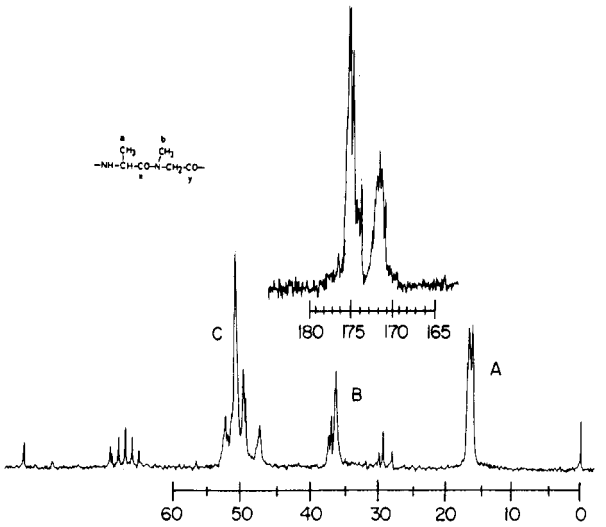

Figure 5. 75.5-MHz ^{13}C NMR spectrum of polypeptide (CP-3) (TFA-D_1).

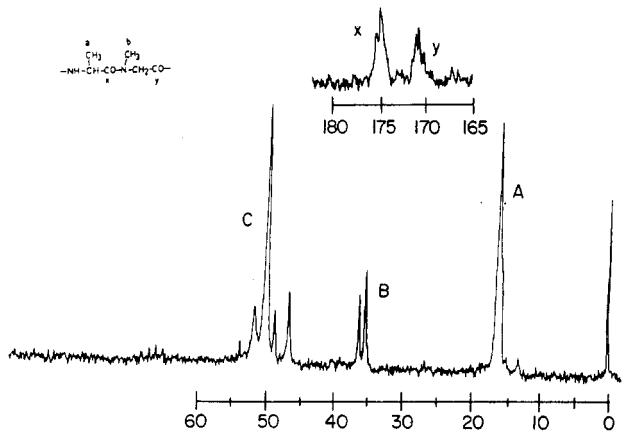

Figure 6. 75.5-MHz ^{13}C NMR spectrum of polypeptide (CP-4) (TFA-D_1).

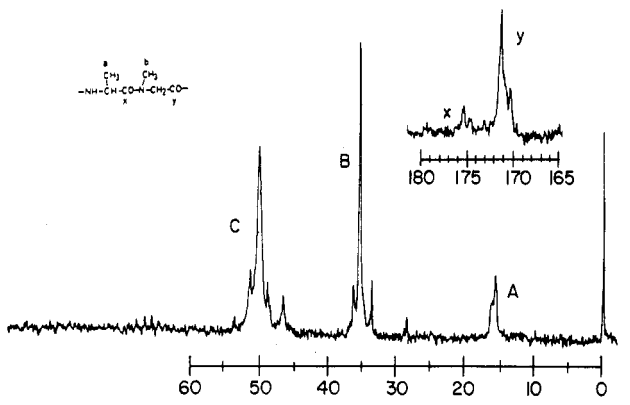

Figure 7. 75.5-MHz ^{13}C NMR spectrum of polypeptide (CP-5) (TFA-D_1).

Figures 4–7 show the ^{13}C NMR spectra of copolypeptides CP-2, CP-3, CP-4, and CP-5. These polymerizations were catalyzed by low molecular weight (P(2VPy) template. As with CP-1, the splitting of all L-Ala signals by the cis/trans isomerism of the *N*-acyl sarcosyl bond indicates that copolypeptides were obtained.

From CP-4 and CP-5 and the fact that the trans *N*-acyl sarcosyl conformation is energetically more favorable,[12] we are able to assign the three carbonyl signals for the L-Ala and Sar residues; see Table III. This was done because Kricheldorf[11] has shown that there is a chemical shift of ca. 1.0 ppm in the carbonyl signals of α-amino acid residues

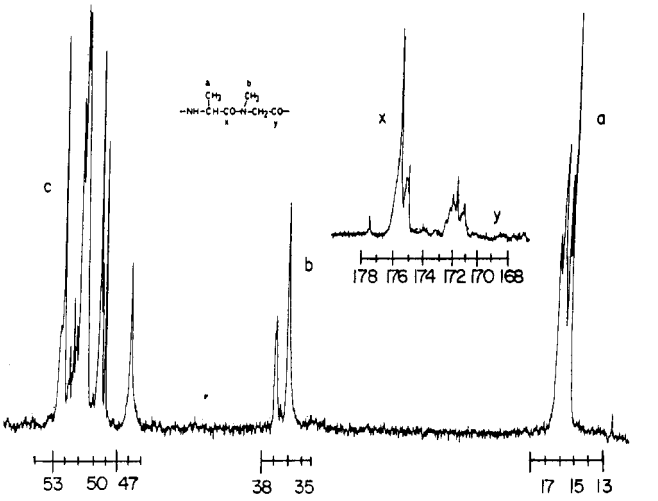

Figure 8. 75.5-MHz ^{13}C NMR spectrum of polypeptide (CP-6) (TFA-D_1).

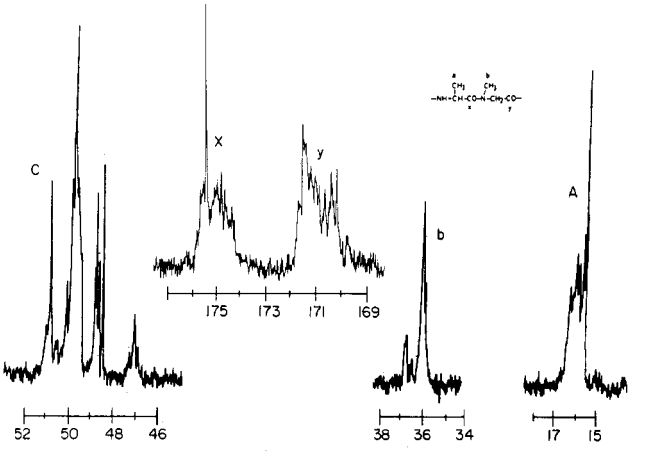

Figure 9. 75.5-MHz ^{13}C NMR spectrum of polypeptide (CP-7) (TFA-D_1).

in copolypeptides. These carbonyl signals along with the β-C of Ala are of the most interest in this case as they are a direct indication of the type of copolypeptides obtained, i.e, random, block, or alternating.

By close inspection of the ^{13}C NMR spectra of CP-2 and CP-3 polypeptides, along with their close resemblance to the spectrum CP-1, it is concluded that random copolypeptides were obtained. CP-2 and CP-3 lack any increase in the Ala–Ala carbonyl signal concomitant with a decrease in the Sar–Ala carbonyl resonance or vice versa and the β-C of L-Ala is still dominated by the cis/trans isomerism of the *N*-acyl sarcosyl bond. This observation leads us to conclude that a copolypeptide was obtained which lacks substantial L-Ala blocks or any specific type of *n*-ad sequence.

Catalysis by High Molecular Weight P(2VPy)

The lack of effect of low molecular weight P(2Vpy) in inducing a blocklike copolypeptide induced us to investigate the effect of a high molecular weight P(2VPy) template. Figures 8–11 are the ^{13}C NMR spectra of copolypeptides CP-6, CP-7, CP-8 and CP-9 which were catalyzed by the high molecular weight P(2VPy) (MW = 20 000).

The spectrum of CP-6 when compared to the previous copolypeptides clearly shows additional fine structure in the L-Ala α-C and β-C region. The carbonyl region for L-Ala exhibits two peaks, with fine splitting of both. This would indicate the L-Ala residues occupy an ordered en-

Table II
75.5-MHz ^{13}C NMR Chemical Shifts δ (relative to Me_4Si) of Polypeptides Measured in Trifluoroacetic Acid at 25 °C

		δ of L-alanine residue, ppm			δ sarcosine residue, ppm		
catalyst	polypeptide	CO	α-C[a]	β-C	CO	α-C[a]	β-C
2-vinylpyridine	$(L\text{-Ala})_n$	175.55	50.65	16.38			
	$(Sar)_n$				171.51	50.74	36.13
					170.43		
	CP-1	175.63	50.59	16.19	171.59	51.90	36.95
		175.04	49.41	15.75	171.08	50.59	36.62
		174.66	49.09		170.51	47.09	35.87
low MW P(2VPy)	CP-2	175.61	50.49	16.19	171.57	51.96	36.95
		175.05	49.30	15.85	171.05	50.48	36.60
		174.69			170.46	46.98	35.87
	CP-3	175.65	50.49	16.19	171.52	51.73	36.93
		175.07	49.25	15.70	171.05	50.49	36.56
							36.02
		174.69			170.48	46.98	35.86
	CP-4	175.61	50.49	16.34	171.37	52.16	36.95
		175.09	49.25		171.00	50.49	36.03
					170.67	47.09	
					170.46		
	CP-5	175.51	50.74	16.16	171.51	51.98	36.88
			49.42	15.85	170.43	50.74	36.07
						46.96	
high MW P(2VPy)	CP-6	175.62	50.50	16.06	171.56	51.79	36.88
		174.71	49.23[b]	15.90	171.09	50.50	35.87
				15.66	170.57	47.00	
				15.51			
	CP-7	175.51	50.55	16.35	171.68	51.84	36.82
		175.02	49.18[b]	16.04	170.58	50.55	36.00
		174.62		15.86	170.40	46.96	35.90
				15.64			
				15.52			
	CP-8	175.69	50.50	16.03	171.81	51.90	36.90
		174.74	49.20[b]	15.88	171.59[b]	50.50	36.08
		174.68		15.64	170.96	47.10	35.90
				15.54	170.58		
	CP-9	175.65	50.50	16.05	171.84	51.90	36.90
		175.02	49.25[b]	15.92	171.51[b]	50.50	36.08
		174.71		15.68	170.96[b]	47.16	
		174.64		15.54	170.59[b]		

[a] Assignment of α-C of Ala and Sar from ref 11. [b] Multiplet center at this chemical shift.

Table III
Chemical Shift of Ala and SAR Carbonyl in Copolypeptides

copolypeptide	δ, ppm
Ala-(trans) Sar	175.62 ± 0.02
Ala-Ala	175.06 ± 0.03
Ala-(cis) Sar	174.67 ± 0.02
Sar-(trans) Sar	171.54 ± 0.03
Sar-Ala	171.05 ± 0.03
Sar-(cis) Sar	170.50 ± 0.05

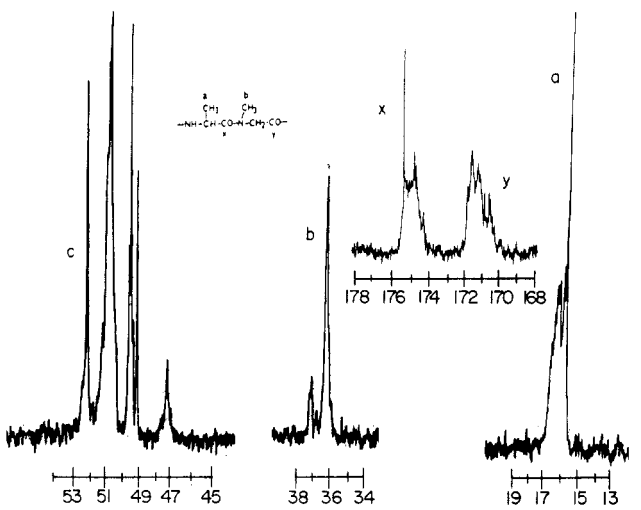

Figure 10. 75.5-MHz ^{13}C NMR spectrum of polypeptide (CP-8) (TFA-D_1).

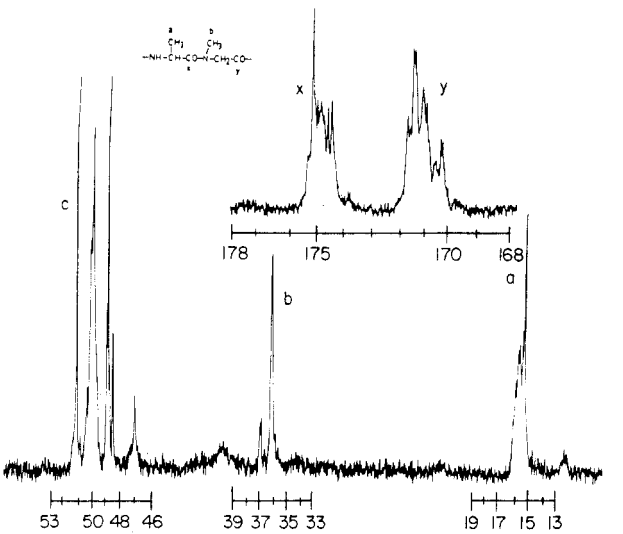

Figure 11. 75.5-MHz ^{13}C NMR spectrum of polypeptide (CP-9) (TFA-D_1).

vironment in the copolypeptide chain. The chemical shifts of the L-Ala carbonyls correspond to Ala–(trans) Sar and Ala–(cis) Sar resonances (see Tables II and III), which indicates a central triad sequence of Sar-Ala-Sar. This triad sequence must be part of a higher n-ad sequence, heptad or higher, as integration yields a Sar/Ala ratio of 1.2:1. The fact that the L-Ala residues nearest neighbors are both Sar needs to be reconciled. If the L-Ala residue

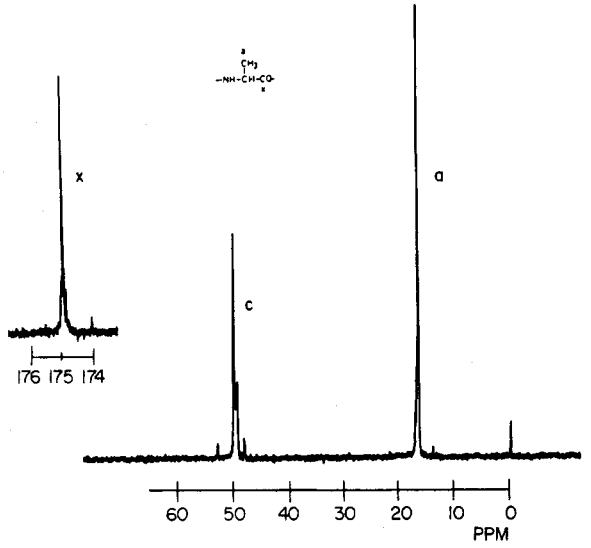

Figure 12. 75.5-MHz ^{13}C NMR spectrum of poly(L-alanine) (TFA-D_1).

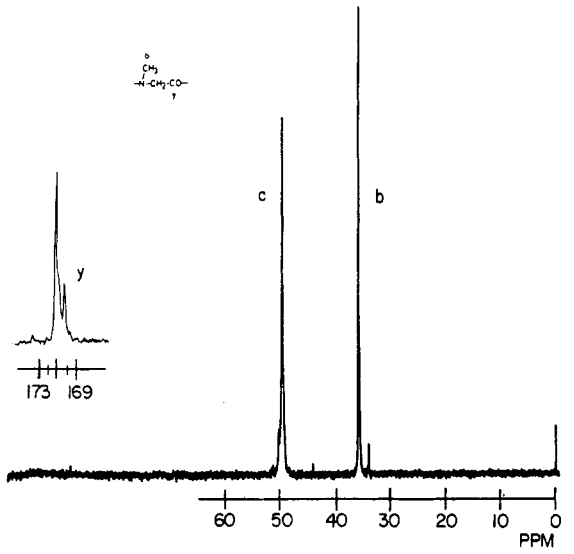

Figure 13. 75.5-MHz ^{13}C NMR spectrum of poly(sarcosine) (TFA-D_1).

is not associated by hydrogen bonding to the P(2VPy) template, copolymerization is taking place in the bulk solution and a random copolypeptide should be obtained as in the case of CP-1. If the L-Ala monomer is in fact associated with the template it could be in an unfavorable position to react with a neighboring L-Ala NCA monomer and requires an intervening Sar residue to put it back in position to react with another L-Ala monomer.

The spectrum of CP-7 is similar to CP-6 in that substantial fine structure is evident for the α-C region of L-Ala and the β-C of L-Ala shows at least five resolvable signals. In contrast, the carbonyl region for L-Ala and Sar shows only one distinct peak, at the downfield end of a broad resonance. The broad Ala–Ala and Sar–Ala resonances and the appearance of a peak at 16.35 ppm, the resonance of the β-C of L-Ala in the homopolypeptide, indicates that small sequences, i.e., tetrad or pentad, of L-Ala residues are present in the copolypeptide. Integration yields an Ala/Sar ratio of 1.3:1, which also indicates small L-Ala blocks. Since the L-Ala resonances are not clearly resolved we are not able to calculate the precise extent to which the short L-Ala blocks are present. However, we can see that they do account for a moderate portion of the copolypeptide chain.

Table IV
Apparent Molecular Weight Averages of Polypeptides

catalyst	poly-peptide	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	dispersity
2-vinylpyridine	CP-1	18.5	22.8	1.23
low MW P(2VPy)	CP-2	16.0	47.3	2.93
	CP-3	17.8	48.6	2.73
	CP-4	26.0	157	6.05
	CP-5	15.5	34.1	2.19
high MW P(2VPy)	CP-6	18.1	54.5	3.01
	CP-7	19.8	55.6	2.81
	CP-8	20.6	56.6	2.75
	CP-9	22.1	59.9	2.71

Finally, Figures 10 and 11 show the ^{13}C NMR spectra of CP-8 and CP-9. The aliphatic region of both are similar. The L-Ala α-C shows fine splitting and the β-C of L-Ala shows four resolvable peaks (a doublet with fine splitting). The Sar carbonyl region consists of four resolvable peaks with fine splitting of all except the shoulder peak at 171.83 ppm. This shoulder peak cannot be assigned at this time but it is related to some type of -Sar-(trans)Sar sequence. The L-Ala carbonyls are similar in the upfield and downfield resonances, but CP-8 lacks any clearly resolvable Ala–Ala resonances. Because of the width of the Ala carbonyl region and the lack of an Ala β-carbon resonance at 16.35 ppm, it is concluded that small blocks of L-Ala are not present in CP-8 and it is most likely a random copolypeptide, as appears also to be the case with CP-9.

Influence of Concentration and Molecular Weight of P(2VPy) on the Apparent Molecular Weight of Copolypeptide. Table IV summarizes the results obtained when L-Ala NCA and Sar NCA were copolymerized with P(2VPy) of two different molecular weights and at various concentrations along with the copolymerization catalyzed with 2-vinylpyridine.

These results indicate (i) the molecular weight of the P(2VPy) has a small effect on the apparent molecular weight of the resulting copolypeptides (compared with the polypeptide catalyzed with 2-vinylpyridine, CP-1, very little effect is noted); (ii) as the concentration of P(2VPy) increases the apparent molecular weight of the copolypeptides increases slightly; (iii) the dispersities of the copolypeptides catalyzed with P(2VPy) are much higher than the dispersity of CP-1.

Although there is no correlation between the molecular weight or concentration of P(2VPy) and the apparent molecular weights of the copolypeptides, the differences in the dispersities indicate that there is a different mechanism of polymerization or termination in the presence of a polymeric template.

Conclusions

Low molecular weight P(2VPy) did not show any template effect on the copolymerization of L-Ala NCA and Sar NCA. However, higher molecular weight P(2VPy) did produce an effect on the copolymerization. Small blocks of Ala were produced in copolypeptide CP-7, which was catalyzed by a 0.50 mol % of P(2VPy) of molecular weight 20 000. The lack of Ala blocks for copolypeptide CP-6, CP-8, and CP-9 is puzzling in that such a narrow concentration range (0.50 mol % but not 0.25, 1.00, or 1.50 mol % P(2VPy)) causes an effect. One would expect that as the concentration increases and the number of available sites for L-Ala NCA to associate with P(2VPy) therefore also increases an increase in template effect would emerge. A more detailed interpretation of the ^{13}C-NMR spectra would require a detailed study of model copolypeptides of known sequence, a project of considerable complexity which must be left for a future study.

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Registry No. P(2VPy), 25014-15-7; (L-ALA NCA)(SARNCA) (copolymers), 108561-50-8; L-ALANCA, 2224-52-4; SARNCA, 5840-76-6.

References and Notes

(1) Szwarc, M. *J. Polym. Sci.* **1954**, *13*, 317.
(2) Ballard, D. G. H.; Bamford, C. H. *Proc. R. Soc. London, A* **1956**, *A236*, 384.
(3) (a) Suzuoki, K.; Imanishi, Y.; Higashimura, T.; Okamura, S. *Biopolymers* **1969**, *7*, 917. (b) Kabanov, V. A.; Aliev, K. V.; Kargina, O. V.; Patrikeeva, T. I.; Kargin, V. A. *J. Polym. Sci., Polym. Symp.* **1967**, *No. 16*, 1079. (c) Ferguson, J.; Shah, S. A. O. *Eur. Polym. J.* **1968**, *4*, 611. (d) Bartels, T.; Tan, Y. Y.; Challa, G. *J. Polym. Sci., Polym. Chem. Ed.* **1977**, *15*, 341.
(4) Suzuoki, K.; Imanishi, Y.; Hagashimura, T.; Okamura, S. *Biopolymers* **1969**, *7*, 925.
(5) Szwarc, M. *Adv. Polym. Sci.* **1965**, *4*, 1.
(6) Leuchs, J. L. *Ber.* **1906**, *39*, 857.
(7) Leuchs, J. L.; Manare, W. *Ber.* **1907**, *40*, 3235.
(8) Idelson, M.; Blout, E. R. *J. Am. Chem. Soc.* **1957**, *79*, 3948.
(9) Natta, G.; Mazzanti, G.; Pong, A.; Dall'asta, G.; Bernardini, F. J. *J. Polym. Sci.* **1961**, *51*, 487.
(10) Kricheldorf, H. R.; Schilling, G. *Makromol. Chem.* **1977**, *178*, 3115.
(11) Kricheldorf, H. R.; Schilling, G. *Makromol. Chem.* **1978**, *179*, 1175.
(12) Flory, P. J. *Statistical Mechanics of Chain Molecules*; *Interscience*: New York, **1969**.
(13) Yau, W. W.; Kirkland, J. J.; Bly, D. D. *Modern Size-Exclusion Liquid Chromotography*; Wiley: New York, 1979.

Determination of Bonding Patterns of ^{13}C Specifically Enriched Dehydrogenatively Polymerized Lignin in Solution and Solid State

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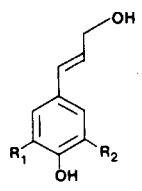
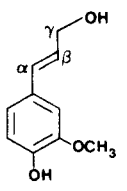
ABSTRACT: Synthetic dehydrogenatively polymerized (DHP) lignins were prepared by the oxidative polymerization of various coniferyl alcohols, enriched with ^{13}C at C_γ, C_β, $C_{\beta\gamma}$, and C_α. The solution and solid-state ^{13}C NMR spectrum of each was then recorded. The solution spectra further confirmed assignments for unlabeled DHP lignin. Solid-state spectra, while subject to extensive line broadening, nevertheless gave valuable information. It was established that the major bonding patterns, namely, substructures containing coniferyl alcohol (A), β-*O*-(4-aryl)glycerol (C), and pinoresinol (D) moieties could readily be distinguished. It was also shown that coniferaldehyde (B) substructures were artifacts. With the labeling methods used it was not possible to distinguish between phenylcoumaran (E) and β-1 (F) moieties. The bonding patterns of a DHP lignin (enriched at C_γ to 4% with ^{13}C) in a cellulose matrix (ratio DHP:cellulose, 24% w/w) could be identified. It therefore seems feasible to examine the bonding patterns of lignin in situ, if specifically labeled lignified tissue enriched to the level of >4% can be obtained.

Introduction

Lignin, a widely distributed vascular plant polymer, is nature's second most abundant naturally occurring organic material. It is generally considered to be formed *exclusively* via the random, dehydrogenative polymerization of the three trans (*E*) monolignols, *p*-coumaryl (**1**), coniferyl (**2**), and sinapyl (**3**) alcohols.[1] This polymerization reaction is thought to be catalyzed by cell-wall-bound peroxidases, whose action on the monomers **1–3**, in the presence of H_2O_2, results in the formation of highly reactive free radical intermediates which combine randomly to give lignin.[2] The ratio of each monomer in the lignin polymer has been shown to be both species[3] and morphological origin[4,5] dependent.



1, $R_1, R_2 = H$
2, $R_1 = H$, $R_2 = OCH_3$
3, $R_1, R_2 = OCH_3$



4a, $C_\alpha = {}^{13}C$
b, $C_\beta = {}^{13}C$
c, $C_\gamma = {}^{13}C$
d, $C_{\beta,\gamma} = {}^{13}C$

Lignin is relatively intractable and isolation procedures, whether chemical or biochemical, are drastic. Irreversible changes to the original lignin macromolecule are therefore *unavoidable*. Lignins are thus classified according to their source, e.g., hardwoods, or softwoods,[6] and method of isolation, e.g., kraft, sulfite, and milled wood lignins.[6] Consequently, the bonding pattern of lignin in situ has never been established.

Much of our current understanding of lignin structure comes from the comparison of isolated lignins with an artificial preparation, known as dehydrogenatively polymerized (DHP) lignin. This material is produced by the in vitro dehydrogenative polymerization of monolignols 1–3; a reaction catalyzed by peroxidase/H_2O_2. DHP lignin is considered to more or less represent native lignin, although differences between it and isolated lignin derivatives have been noted.[7,8] In the absence of a better model, DHP lignin

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