(7) A. Levitzki, I. Pecht, and A. Berger, *J. Am. Chem. Soc.*, **94**, 6844 (1972).
(8) A. Garnier and L. Tosi, *Biopolymers*, **14**, 2247 (1975).
(9) A. Garnier and L. Tosi, *Biochem. Biophys. Res. Commum.*, **74**, 1280 (1977).
(10) R. E. Wasylishen and J. S. Cohen, *J. Am. Chem. Soc.*, **99**, 2480 (1977).
(11) M. Palumbo, A. Cosani, M. Terbojevich, and E. Peggion, *J. Am. Chem. Soc.*, **99**, 943 (1977).
(12) M. Palumbo, A. Cosani, M. Terbojevich, and E. Peggion, *Macromolecules*, **10**, 813 (1977).
(13) M. Palumbo, A. Cosani, M. Terbojevich, and E. Peggion, *Biopolymers*, **17**, 243 (1978).
(14) D. R. Eaton and W. D. Phillips, *Adv. Magn. Reson.*, **1**, 103 (1965).
(15) A. S. Mildvan and M. Cohn, *Adv. Enzymol.*, **33**, 1 (1970).
(16) G. N. La Mar, W. D. Horrocks, and R. H. Holms, Eds., "NMR of Paramagnetic Molecules", Academic Press, New York, 1973.
(17) R. A. Dwek, "Nuclear Magnetic Resonance in Biochemistry", Clarendon Press, Oxford, 1973, Chapters 9 and 10.
(18) O. Iwaki, K. Hikichi, M. Kaneko, S. Shimizu, and T. Maruyama, *Polym. J.*, **4**, 623 (1973).
(19) T. Hiraoki, M. Kaneko, and K. Hikichi, *Polym. J.*, **11**, 397 (1979).
(20) T. Hiraoki, A. Tsutsumi, and K. Hikichi, *Polym. J.*, **11**, 591 (1979).
(21) K. F. Kuhlmann, D. M. Grant, and R. K. Harris, *J. Chem. Phys.*, **52**, 3439 (1970).
(22) S. J. Opella, D. J. Nelson, and O. Jardetzky, *J. Chem. Phys.*, **64**, 2533 (1976).
(23) T. J. Swift and R. E. Connick, *J. Chem. Phys.*, **37**, 307 (1962).
(24) Z. Luz and S. Meiboom, *J. Chem. Phys.*, **40**, 2686 (1964).
(25) I. Solomon, *Phys. Rev.*, **99**, 559 (1955).
(26) N. Bloembergen, *J. Chem. Phys.*, **27**, 527 (1957).
(27) E. M. Bradbury, C. Crane-Robinson, H. Goldman, and E. W. W. Rattle, *Biopolymers*, **6**, 851 (1968).
(28) R. A. Bernheim, T. H. Brown, H. S. Gutowsky, and D. E. Woessner, *J. Chem. Phys.*, **30**, 950 (1958).
(29) A. Abragam, "The Principle of Nuclear Magnetism", Clarendon Press, Oxford, 1961, Chapter 8.
(30) H. Saito and I. C. P. Smith, *Arch. Biochem. Biophys.*, **158**, 154 (1973).
(31) T. Hiraoki, Doctoral thesis, Hokkaido University, 1979.
(32) N. Bloembergen and L. O Morgan, *J. Chem. Phys.*, **34**, 842 (1961).
(33) G. N. La Mar, *J. Am. Chem. Soc.*, **93**, 1040 (1971).
(34) R. Freeman, K. G. R. Pachler, and G. N. La Mar, *J. Chem. Phys.*, **55**, 4586 (1971).
(35) D. F. S. Natush, *J. Am. Chem. Soc.*, **93**, 2566 (1971).
(36) R. A. Dwek, "Nuclear Magnetic Resonance in Biochemistry", Clarendon Press, Oxford, 1973, Chapter 11.
(37) N. Higuchi, K. Kakiuchi, Y. Kyogoku, and K. Hikichi, *Macromolecules*, preceding paper in this issue.
(38) E. Peggion, A. Cosani, M. Palumbo, and M. Terbojevich, *Pept.: Chem. Biochem., Proc. Am. Pept. Symp.*, 5th, 1977, 371 (1977).

^{15}N NMR Spectroscopy. 21.[1] Copolymerization of Glycine *N*-Carboxyanhydride with γ-Methyl Glutamate *N*-Carboxyanhydride, *S*-Benzylcysteine *N*-Carboxyanhydride, Leucine *N*-Carboxyanhydride, and Valine *N*-Carboxyanhydride

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ABSTRACT: The copolymerization of Gly-NCA with γ-MeGlu-NCA, *S*-BzlCys-NCA, Leu-NCA, and Val-NCA was investigated in various solvents but mostly in dioxane. Primary, secondary, and tertiary amines were used as initiators, and in some cases an electrophilic cocatalyst, e.g., 4-chlorophenyl isocyanate, was added. The 36.48 MHz natural abundance ^{15}N NMR spectra of the resulting copolypeptides, measured in trifluoroacetic acid, display four signals which represent the four different types of peptide groups which can exist in binary copolypeptides. A combined, quantitative evaluation of the ^{1}H NMR and ^{15}N NMR spectra allows one to calculate the average length of the homogeneous blocks and the copolymerization parameters. This evaluation demonstrates that the nature of the sequences depends mainly on the reactivity of the monomers, which decreases in the following order: Gly-NCA > γ-MeGlu-NCA > *S*-BzlCys-NCA > Leu-NCA > Val-NCA. While in most cases random sequences are predominantly formed, the copolymerization of Gly-NCA with Leu-NCA and Val-NCA shows a tendency to yield block copolymers.

The copolymerization of α-amino acid NCA's allows the synthesis of copolypeptides of all common α-amino acids. Since the ratio of the monomer units and the molecular weights can be varied over a wide range, these synthetic copolypeptides are useful model compounds for proteins. Chemical, physicochemical, and physical studies on the properties of such copolypeptides require that the primary structure is known, because most properties depend on the sequence. However, the enzymatic, chemical, and physical methods widely used for the sequence analysis of proteins cannot be applied to synthetic copolypeptides. Thus, spectroscopic methods useful for the characterization of peptide sequences are desirable, in as much as they are nondestructive, time saving, and routine. Since ^{1}H NMR spectra of copolypeptides do not contain sequential information, we have studied the ^{13}C NMR spectra of various copolypeptides; however, only in a few special cases of alanine containing binary copolypeptides is the ^{13}C NMR method successful, even if a magnetic field of 84 kG (8.4 T) is used. Since the ^{17}O spectra of oligopeptides display only extremely broad signals without fine structure,[2] only ^{15}N NMR spectroscopy is possibly useful for a spectroscopic sequence analysis of synthetic copolypeptides. A first success in this direction was achieved when copolymers obtained from Gly-NCA and β-Ala-NCA were spectroscopically analyzed.[3a] The present work was undertaken to test whether copolypeptides exclusively built up from α-aminoacyl units are also accessible to ^{15}N NMR

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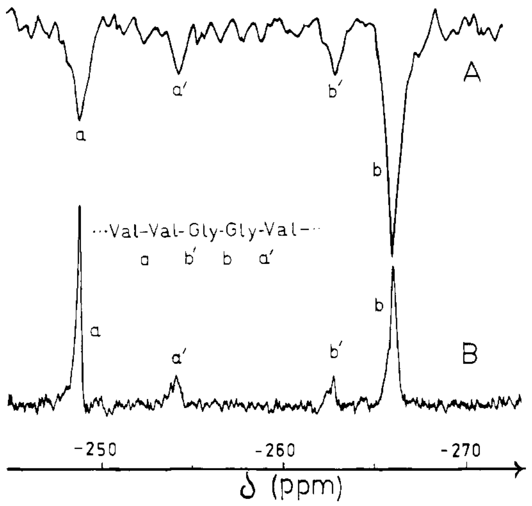


Figure 1. Natural abundance ^{15}N NMR spectra of the copolypeptide (Gly/Val) from experiment no. 7, Table IV, dissolved in TFA: (A) 9.12 MHz spectrum, continuous broad band ^{1}H decoupling, pulse width 40 μs (ca. 40°), 1K data points/500 Hz spectral width, exponential line broadening 1.5 Hz, 50000 transients; and (b) 36.48 MHz spectrum, inverse gated ^{1}H decoupling, pulse width 45 μs (ca. 70°), 2K data points/1200 Hz, delay time 2.5 s, exponential line broadening 2 Hz, 25000 transients.

sequence analysis. A routine application of the method should then allow one to investigate the influence of polymerization conditions on the sequence of the resulting copolypeptides.

Experimental Section

Solvents. Dioxane, diethyl ether, and triethylamine were refluxed and distilled over sodium wire. Dimethylformamide and dimethylacetamide were refluxed and distilled over P_4O_{10} in vacuo; methylene chloride and chloroform were refluxed and distilled over P_4O_{10} under normal pressure. Primary and secondary amines as well as pyridine were refluxed and distilled over freshly powdered calcium hydride.

Amino Acid NCA's. Gly-NCA was prepared from *N*-benzyloxycarbonyl glycine trimethylsilyl ester and distilled thionyl chloride in boiling chloroform.[3b] It was recrystallized three times from tetrahydrofurane/chloroform. For the first and second time the tetrahydrofurane solution was treated with dry charcoal. Leu-NCA, Val-NCA, *S*-BzlCys-NCA, and γ-Me-Glu-NCA were obtained by phosgenation of the corresponding amino acids in a refluxing 1:1 mixture (by volume) of dry dioxane and dry methylene chloride. These monomers were recrystallized twice, and the NCA solution was treated the first time with dry charcoal.

Copolymerizations. Quantities of each monomer (40 mmol) were dissolved together in 100 mL of solvent, and the catalyst was added in the form of a 1 M solution in dry dioxane. The reaction flasks were protected by freshly prepared calcium chloride drying tubes. The polypeptides were precipitated from ca. 800 mL of diethyl ether, washed with diethyl ether, and dried at 70 °C (12 mbar) for at least 24 h. In the case of experiments no. 11, Table II, and no. 7, Table IV, the solid monomers were mixed and heated under nitrogen. The resulting solid foam of the copolypeptide was powdered, dissolved in a mixture of 20 mL of trifluoroacetic acid and 60 mL methylene chloride, and precipitated from 800 mL of diethyl ether.

Measurements. The 36.48 MHz natural abundance ^{15}N NMR spectra were measured on a Bruker WH-360 (magnetic field strength 84 kG = 8.4 T) at 30 °C. For all spectra the inverse-gated ^{1}H decoupling technique and the same acquisition parameters were used (Figure 1B). One gram of copolypeptide dissolved in 4–5 mL of trifluoroacetic acid (TFA) was measured in 15-mm diameter sample tubes. A coaxial 5-mm tube containing D_2O served for lock purposes and the NO_3^- ion of a $^{15}NH_4$–$^{15}NO_3$ solution in D_2O for shift referencing. The 9.12 MHz ^{15}N NMR spectrum of Figure 1A was obtained on a Bruker WH-90 (magnetic field strength 21 kG = 2.1 T) at 30 °C with continuous broad-band ^{1}H decoupling. Copolypeptide (1.5 g) dissolved in 7 mL of trifluoroacetic acid was measured in a 20-mm diameter sample tube with a coaxial 5-mm tube containing D_2O for lock purposes. The NOE measurements were carried out with 1.2 g of $(Gly)_n$ containing 10% ^{15}N in 9 mL of TFA at 30–32 °C in 20-mm sample tubes with a coaxial 5-mm tube containing D_2O. A pulse width of 40 μs (ca. 40°), 1K data points/600 Hz spectral width, and a delay time of 6.0 s (in the case of gated ^{1}H decoupling) were used, and 1000 transients were accumulated.

The ^{1}H NMR spectra were measured with a Bruker WH-90 in 5-mm sample tubes. Solutions of 60 mg of copolypeptide in 0.6 mL of deuterated TFA were used, and internal Me_4Si served for shift referencing. Neighboring residue effects were not found in these ^{1}H NMR spectra.

Results and Discussion

Spectroscopic Conditions for ^{15}N NMR Sequence Analysis. A spectroscopic sequence analysis requires (I) that the signals of the individual monomer units are well resolved and assigned and (II) that the signal intensities reflect the concentration of the monomer units in the copolymer. Figure 1A demonstrates that condition I is already fulfilled for a glycine and valine containing copolypeptide when the 9.12 MHz ^{15}N NMR spectra are measured. Figure 5 shows, on the other hand, that for a glycine/leucine copolypeptide a magnetic field strength >21 kG (2.1 T) is necessary to obtain satisfactory resolution. However, even in cases where resolution is not the limiting factor, spectra measured at a frequency of 9.12 MHz are not useful for the ^{15}N NMR sequence analysis because of an unfavorable combination of nuclear Overhauser effect (NOE) and signal-to-noise ratio. When the NOE is eliminated by applying the inverse-gated ^{1}H decoupling technique, the signal-to-noise ratio is too low for a quantitative evaluation of the spectra even with data accumulation over a period of 24 h. With continuous broad-band ^{1}H decoupling, an acceptable signal-to-noise ratio is achievable; however, condition II is never fulfilled because blocks of aminoacyl units with bulky side chains have a lower NOE than those with small substituents. Thus, the signal intensity of the $(Val)_n$ block in Figure 1A is only ca. 60% of that of the $(Gly)_n$ block, although ^{1}H and ^{13}C NMR spectra indicate that the Gly–Val ratio in this copolypeptide is 1.1/1 (see experiment no. 7, Table IV). Thus, we can conclude that the NOE of the $(Val)_n$ blocks in the copolypeptide of Figure 1A is only 60–70% of that of the $(Gly)_n$ blocks. At 9.12 MHz comparing the inverse-gated decoupling technique with a continuous broad band decoupling, we found a NOE of −3.3 to −3.5 for two polyglycine samples with polymerization degrees in the range of 50–90, measured under the same conditions as that of the copolypeptide of Figure 1A. Hence, the NOE of the $(Val)_n$ blocks are in the range of −2.0 to −2.5. According to the NOE–correlation time (τ_c) relationship calculated for 9.12 MHz spectra, the NOE values correspond to effective isotopic correlation times of ca. 0.8×10^{-10} s for $(Gly)_n$ blocks and ca. 2.0×10^{-9} s for the $(Val)_n$ blocks (Figure 2). Thus, small differences in segmental mobility cause large differences in the NOE for correlation times in the range 10^{-8}–10^{-9} s. Consequently, signal intensities reflecting the mole ratio of the monomer units are only expected for magnetic fields <0.7 T or >20 T, because then polypeptides of all common amino acids should show the maximum NOE (−3.9) or the minimum NOE (+0.9). However, at a magnetic field strength of <0.7 T resolution would never by sufficient, and 20-T magnets are currently not available. Hence, only the currently available superconducting NMR spectrometer with field strength in the range 4.2–9.4 T and the application of the inverse-gated ^{1}H decoupling technique allow a satisfactory ^{15}N NMR sequence analysis of copolypeptides (Figure 1B). Mea-

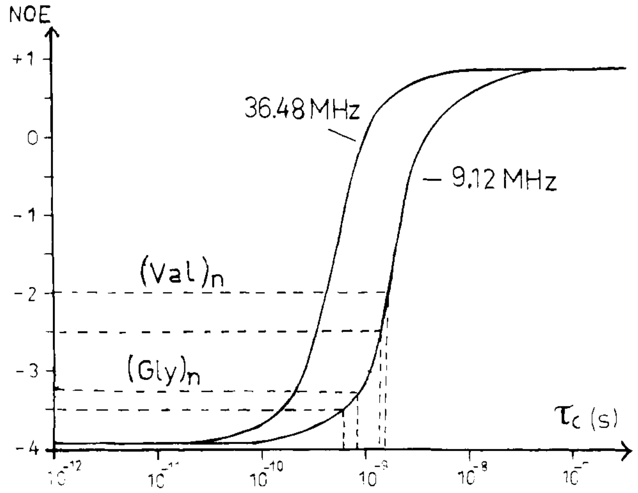


Figure 2. Calculated ^{15}N {^{1}H} NOE enhancement factor (signal intensity with continuous decoupling/signal intensity with inverse gated decoupling) as a function of the isotropic rotational correlation time τ_c for two resonance frequencies. Dashed lines indicate the estimated "effective" τ_c corresponding to the NOE observed at 9.12 MHz for $(Gly)_x$ and $(Val)_x$ blocks in poly(Gly/Val).

surements of other copolypeptides have shown that the problems discussed here for $(Gly/Val)_x$ exist likewise for other copolypeptides of glycine and α-amino acids with space filling side chains, such as leucine, N^ϵ-Z-lysine, γ-methyl glutamate, or S-benzylcysteine. Copolypeptides of glycine and β-alanine units are devoid of side chains. In this case the segmental mobilities of $(Gly)_n$ and $(Ala)_m$ blocks are nearly identical, and continuous broad-band ^{1}H decoupled ^{15}N NMR spectra are useful for the sequence analysis.[2] This is also true for copolypeptides of valine and leucine since the bulkiness of the side chains of both amino acids is comparable. A ^{15}N NMR investigation on the copolymerization of Val-NCA and Leu-NCA will be reported in part 25 of this series.

Quantitative Evaluation of the Spectra. An NMR spectroscopic sequence analysis of a binary copolypeptide can in principle follow two paths. If the NMR spectra only allow one to determine quantitatively the ratio of the two monomer units A and B, no sequence information is obtainable from a single copolypeptide. However, if several copolymerizations are carried out with the same pair of monomers at various mole ratios but under identical reaction conditions, then eq 1 can be used to calculate the

$$\frac{[A]}{[B]} = \frac{1 + r_A([A']/[B'])}{1 + r_B([B']/[A'])} \tag{1}$$

copolymerization parameters r_A and r_B. This kind of sequence analysis follows in principle the method first used by Mayo and Lewis for their investigation of the radical copolymerization of vinyl monomers[5] and, thus, will be called here the "classical method". This kind of sequence analysis is achievable for all binary copolypeptides by means of high-resolution ^{1}H NMR spectroscopy, because only one signal of A and one signal of B must be well resolved to allow a quantitative determination of the [A]/[B] ratio {[A], [B] are the mole concentrations of the monomer units A and B in the copolymer (relative to the molar sum of A and B), and [A′], [B′] are the mole concentrations of the monomers A′ and B′ in the reaction mixture}.

^{15}N NMR spectra (36.48 MHz) of many copolypeptides offer the advantage that the four different kinds of peptide linkages present in a binary copolypeptide show individual signals (Figures 1 and 3–6). Two of these signals represent the homogeneous blocks or in other words the A–A and B–B bond. They can be easily assigned by comparison with the signals of the corresponding homopolypeptides, as long as both homo- and copolypeptides possess identical secondary structures in solution. The signals of the A–B

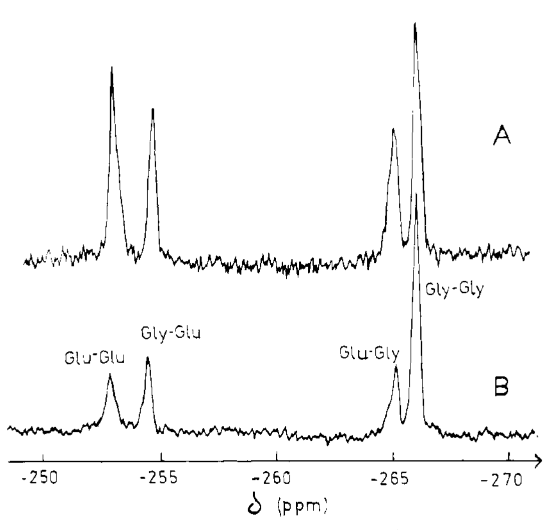


Figure 3. Natural abundance 36.48 MHz ^{15}N-NMR spectra of $(Gly/\gamma\text{-}MeGlu)_n$ in TFA, measured with inverse gated ^{1}H decoupling: (A) experiment no. 3, Table I (40 000 transients); and (B) experiment no. 2, Table I (22 000 transients).

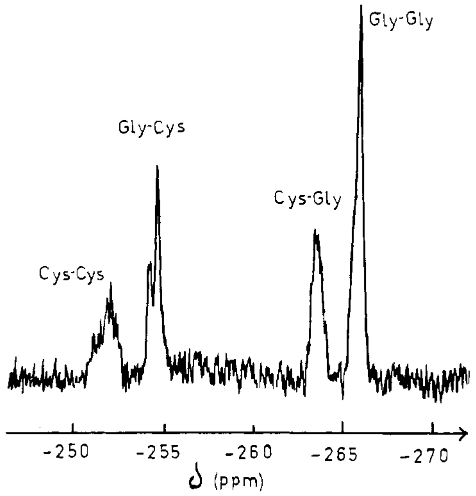


Figure 4. Natural abundance 36.48 MHz ^{15}N-NMR spectrum of $(Gly/S\text{-}BzlCys)_n$ from experiment no. 7, Table II, in TFA, measured with inverse gated ^{1}H decoupling (20 000 transients).

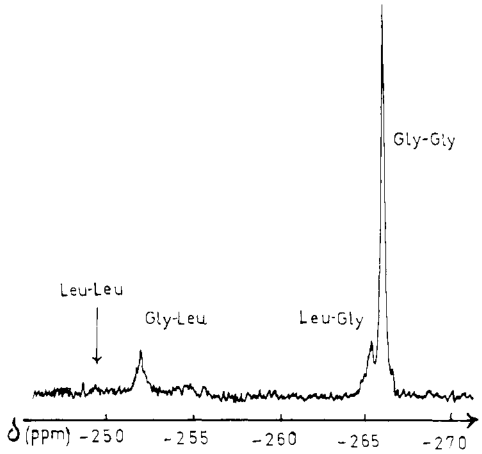


Figure 5. Natural abundance 36.48 MHz ^{15}N NMR spectrum of $(Gly\text{-}Leu)_n$ from experiment no. 4, Table III, in TFA, measured with inverse gated ^{1}H decoupling (15 000 transients).

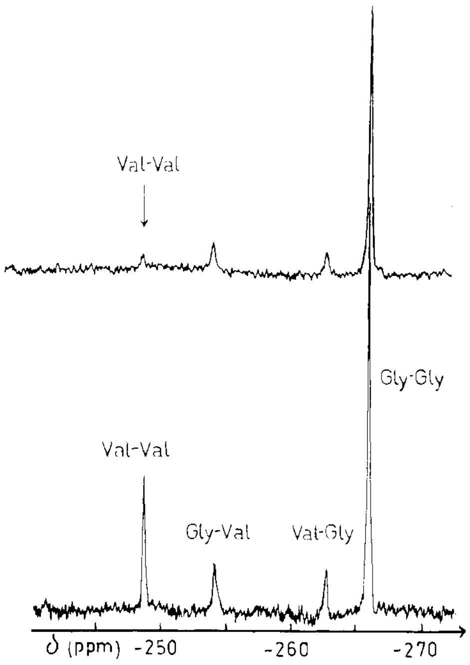


Figure 6. Natural abundance 36.48 MHz ^{15}N NMR spectra of (Gly/Val)$_n$ in TFA, measured with inverse gated ^{1}H decoupling: (A) experiment no. 1, Table IV (20 000 transients); and (B) experiment no. 6, Table IV (20 000 transients).

and B–A bonds are characterized by equal intensities, because the reaction between the active chain end A and monomer B′ and the reaction between chain end B and monomer A′ must have equal ratios. From the intensity ratios of the four peptide signals three kinds of parameters characterizing the sequence can be calculated. These parameters are (1) the average length of the homogeneous blocks computable from eq 2, (2) the copolymerization

$$\bar{L}_A = (I_{AA}/I_{BA}) + 1; \qquad \bar{L}_B = (I_{BB}/I_{AB}) + 1 \qquad (2)$$

parameters (or reactivity ratios) which are directly obtainable from the signal intensities of one single sample according to eq 3, and (3) the run number, previously

$$\begin{aligned} \frac{V_{AA}}{V_{AB}} &= \frac{k_{AA}[A']}{k_{AB}[B']} = \frac{I_{AA}}{I_{BA}};\ r_A = \frac{k_{AA}}{k_{AB}} = \frac{I_{AA}[B']}{I_{AB}[A']} \\ \frac{V_{BB}}{V_{BA}} &= \frac{k_{BB}[B']}{k_{BA}[A']} = \frac{I_{BB}}{I_{AB}};\ r_B = \frac{k_{BB}}{k_{BA}} = \frac{I_{BB}[A']}{I_{BA}[B']} \end{aligned} \qquad (3)$$

defined by Haarwood,[5] which can be calculated either by eq 4 or 5. The following definitions are used for these

$$R = \frac{200}{2 + r_A[A]/[B] + r_B[B]/[A]} \qquad (4)$$

$$R = \frac{100}{\bar{L}_A} = \frac{100}{\bar{L}_B} \text{ (only for [A] = [B])} \qquad (5)$$

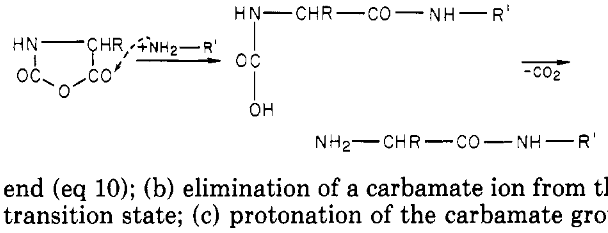
equations: I_{AA}, I_{BB}, I_{AB}, I_{BA} = the signal intensities of the peptide bonds A–A, B–B, A–B, B–A; V_{AA}, V_{BB}, V_{AB}, V_{BA} = the reaction rates of the four growing steps leading to the peptide linkages A–A, B–B, B–A, A–B; and k_{AA}, k_{BB}, k_{AB}, k_{BA} = the reaction constants of the four growing steps. Thus, the ^{15}N NMR sequence analysis allows a rapid and simple determination of several parameters characterizing the copolymerization for two NCA's and the sequence of the resulting copolypeptide. Of course, the accuracy of the I, r, and R values is dependent on the signal-to-noise ratio, and therefore it is not better than ±0.07, when natural abundance ^{15}N NMR spectra are measured. The low signal-to-noise ratio would seriously affect the usefulness of ^{15}N NMR sequence analysis if a combination with the ^{1}H NMR spectroscopy did not expand the range of application. As mentioned, the [A]/[B] ratio of all copolypeptides is easily and accurately measurable by ^{1}H NMR spectra, and this ratio equals that of the block lengths (eq 6). This relationship has valuable applications.

$$([A]/[B])(^1\text{H NMR}) = (\bar{L}_A/\bar{L}_B)(^{15}\text{N NMR}) \qquad (6)$$

First the accuracy of the ^{15}N NMR measurements can be checked by the more accurate ^{1}H NMR spectra. Second, one block length (e.g., $\bar{L}_B$) and the corresponding copolymerization parameter (r_B) can be calculated even if the corresponding ^{15}N NMR signal (I_{BB}) is not directly measurable. This is the case when monomers of different reactivities (e.g., $r_A > 1$, $r_B < 1$) are copolymerized, so that the conversion is kept below 50–70%. Such an experiment leads to a copolymer with a low, eventually not measurable concentration of B–B bonds, as demonstrated by Figures 5 and 6A (experiments no. 4, 5, and 11, Table III, and no. 1–5, Table IV). Another case, which requires the application of eq 5, results from ^{15}N NMR spectra, where the signal of one of the homogeneous blocks (e.g., I_{BB}) is very broad while the other signal (I_{AA}) is relatively sharp. The calculation of $\bar{L}_B$ from $\bar{L}_A$ and eq 5 is then more accurate than the direct measurement of L_B, a situation found in experiment no. 1 and 3, Table II, and no. 1, 3, and 10, Table III.

Limits of the ^{15}N NMR Sequence Analysis. The accuracy of the copolymerization parameters determined from ^{15}N NMR spectra according to eq 3 or calculated by the classical methol 1 is affected by a kinetic feature of the NCA polymerization. The polymerization degree of the polypeptides depends more or less on the conversion, no matter what kind of catalysis was used.[6-8] If the two copolymerization parameters are different, the composition of the copolypeptide ([A]/[B]) depends strongly on the conversion. Hence, the accuracy of the copolymerization parameters must increase with decreasing conversion (for this reason, those r values calculated from high conversions are given in brackets in Tables I–IV). However, in experiments with conversions below 15%, a substantial fraction of the reaction products are oligopeptides which may vary in their solubilities. In this case, precipitation can lead to fractionation and to a partial crystallization of the least soluble, unreacted NCA which finally undergoes postpolymerization. This conflict between theoretical advantage and experimental problems of low conversion does not exist in radical copolymerization of vinyl monomers to which the classical method was originally applied because the radical polymerization of vinyl monomers yields high polymerization degrees even at very low conversions.

Another problem inherent in the NCA polymerization is the complexity of the polymerization mechanism. Each growing step is a sequence of three or four individual reaction steps: (a) addition of a nucleophilic (amino chain end, carbamate chain end or NCA anion) onto the carbonyl group C-5 of a NCA (eq 7 and 11) or N-acyl-NCA chain end (eq 10); (b) elimination of a carbamate ion from this transition state; (c) protonation of the carbamate group



(7)

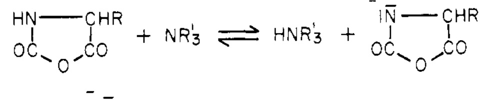
Table I
Reaction Conditions and Results of the Copolymerization of Gly-NCA with γ-Me-L-Glu-NCA

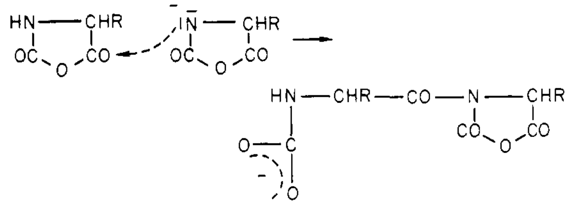
no.	Gly-NCA/ Glu-NCA[a]	cat. + cocat.	NCA[a] catal.	solvent	temp, °C	time, h	yield, %	Gly/Glu[b]	block lengths		reactiv ratio[b]	
									Gly	Glu	Gly	Glu
1	1/1	benzylamine	85/1	dimethylformamide	20	4	42	1.0/1	2.6	2.5	1.6	1.6
2	1/1	benzylamine	85/1	dimethylformamide	20	24	85	1.0/1	2.7	2.6	(1.6)	(1.6)
3	2/1	benzylamine	85/1	dimethylformamide	20	24	86	2.1/1	4.1	1.9	(1.6)	(1.5)
4	1/1	diethylamine	100/1	dimethylformamide	20	24	63	1.0/1	2.4	2.3	(1.4)	(1.3)
5	1/1	diisopropylamine	100/1	dimethylformamide	20	24	29	1.0/1	2.3	2.2	1.3	1.2
6	1/1	triethylamine	100/1	dioxane	20	24	31	2.3/1	3.5	1.5	2.5	0.5
7	1/1	triethylamine	100/1	dimethylformamide	20	24	25	1.2/1	2.6	2.1	1.6	1.1
8	1/1	triethylamine + CPI	100/1	dimethylformamide	20	4	24	1.0/1	2.0	2.1	1.0	1.1
9	1/1	triethylamine + CPI	100/1	dimethylformamide	20	24	74	0.9/1	2.1	2.3	(1.1)	(1.3)

[a] Mole ratios. [b] Margin of error: ca. ±0.07 for values <2.0 and ±0.15 for values >2.0. [c] 4-Chlorophenyl isocyanate (NCA/CPI = 80:1).

(except in the case of the carbamate mechanism (eq 11); and (d) decarboxylation.

Which of these individual reaction steps is rate determining is not yet clear and may be different for the various reaction mechanisms. Futhermore, it must be considered that two different propagation mechanisms can contribute to the chain growth more or less simultaneously. For example, the primary and secondary amine-initiated polymerization can initially proceed mainly via carbamate chain ends (eq 11) until the initiator which stabilizes the carbamate groups is consumed by the start reaction (eq 7). Afterwards, the polymerization will proceed predominantly via amino chain ends. In the case of the base-initiated NCA polymerization, an initial chain growth involving NCA anions and *N*-acyl-NCA chain ends ("activated monomer mechanism", eq 8–10) can be paralleled or followed by a propagation via carbamate chain ends (eq 11) because the starting reaction 9 produces electrophilic and nucleophilic chain ends and because the electrophilic *N*-acyl-NCA chain ends disappear in the course of the polymerization.[23] In addition to that, it must be taken into account that the rate constants k_{AA} and k_{BB} of copolymerization are in most cases not identical with those of the homopolymerization of monomer A or B, even if only one propagation mechanism is operating. The reason for this is the heterogeneous course of nearly all NCA polymerizations. First, the polymerization degree at which the growing oligopeptides precipitate from the reaction medium can be different for homo- and copolypeptides. Since the steric environment of the active chain end in the precipitated material is different from that in the dissolved oligomers, precipitation can markedly influence the reaction rate. Second, the secondary structure which is well known to affect the polymerization rate[19] may be different for homo- and copolypeptides of the same monomers. Finally, it should be pointed out that in the case of the activated monomer mechanism 8–10 the rate


(8)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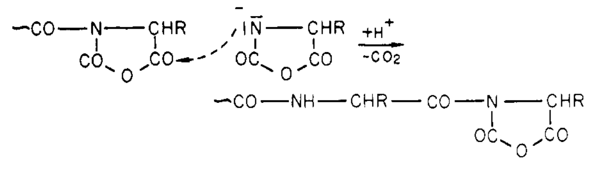
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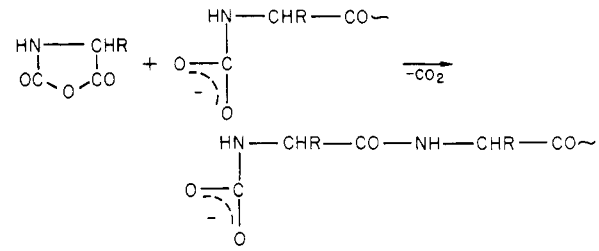
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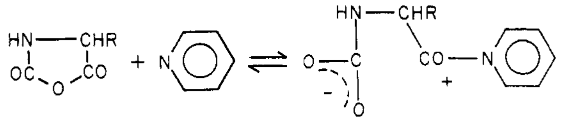
(12)

Table II
Reaction Conditions and Results of the Copolymerization of Gly-NCA with *S*-Bzl-L-Cys-NCA

no.	Gly-NCA/ Cys-NCA[a]	cat. + cocat.	NCA/ catal.[a]	solvent	temp, °C	time, h	yield, %	Gly/Cys[b]	block lengths Gly	block lengths Cys	reactiv ratios[b] Gly	reactiv ratios[b] Cys
1	2/1	*tert*-butylamine	80/1	DMF[d]	20	24	66	2.4/1	3.5	1.5	1.3	1.0
2	1/1	*tert*-butylamine	80/1	DMF[d]	20	24	50	1.2/1	2.3	1.9	1.3	0.9
3	1/1	diethylamine	80/1	DMF[d]	20	24	61	1.6/1	2.8	1.8	1.8	0.8
4	1/1	triethylamine	80/1	DMF[d]	20	24	23	1.0/1	2.1	2.0	1.1	1.0
5	1/1	triethylamine + CPI[c]	80/1	DMF[d]	20	4	30	1.0/1	2.0	2.0	1.0	1.0
6	1/1	triethylamine + CPI[c]	80/1	DMF[d]	20	24	89	1.0/1	2.0	2.0	(1.0)	(1.0)
7	1/1	pyridine	80/1	pyridine + dioxane	20	4	24	1.9/1	3.0	1.6	2.0	0.6
8	1/1	pyridine	80/1	pyridine + dioxane	20	24	69	1.6/1	2.8	1.7	(1.8)	(0.7)
9	1/1	pyridine + CPI	80/1	pyridine + dioxane	20	4	25	1.9/1	2.9	1.6	1.9	0.6
10	1/1	pyridine + CPI	80/1	pyridine + dioxane	20	24	70	1.6/1	2.7	1.8	(1.7)	(0.8)
11	1/1				120	1	58	1.1/1	2.2	2.0	1.2	1.0

[a] Mole ratios. [b] Margin of error: ca. ±0.07 for values <2.0 and ±0.1–0.15 for values >2.0. [c] 4-Chlorophenyl isocyanate (NCA/CPI = 80:1). [d] DMF = dimethylformamide.

Table III
Reaction Conditions and Results of the Copolymerization of Gly-NCA with L-Leu-NCA

no.	Gly-NCA/ Leu-NCA[a]	cat. + cocat.	NCA's[a] catal.	solvent	temp, °C	time, h	yield, %	Gly/Leu[b]	block length[b] Gly	block length[b] Leu	reactiv ratios Gly	reactiv ratios Leu
1	1/1	benzylamine	80/1	dioxane	20	3	41	3.2/1	5.2	1.6	4.2	0.6
2	1/1	benzylamine	80/1	dioxane	20	24	91	1.2/1	4.1	3.3	(3.0)	(3.2)
3	1/2	triethylamine	100/1	dioxane	20	24	21	1.7/1	2.4	1.4	2.8	0.2
4	2/1	triethylamine	100/1	dioxane	20	24	32	4.5/1	5.8	1.2	2.9	0.2
5	1/1	triethylamine	100/1	dioxane	20	24	35	3.4/1	6.0	1.7	5.0	0.7
6	1/1	triethylamine	100/1	dimethylacetamide	20	3	29	1.6/1	3.1	1.9	2.1	0.9
7	1/1	triethylamine	100/1	dimethylacetamide	20	24	35	1.8/1	3.3	1.8	2.3	0.8
8	1/1	triethylamine + CPI[c]	100/1	dimethylacetamide	20	24	78	1.4/1	2.9	2.1	(1.9)	(1.1)
9	1/1	pyridine	1/20	pyridine	20	3	55	2.2/1	4.1	1.9	3.1	0.9
10	1/1	pyridine	1/20	pyridine	20	24	91	1.2/1	3.4	2.7	(2.4)	(1.7)
11	1/1	pyridine + CPI	1/20	pyridine	20	0.5	16	4.6/1	6.0	1.3	5.0	0.3
12	1/1	pyridine + CPI	1/20	pyridine	20	3	47	2.8/1	4.7	1.7	(3.6)	(0.7)
13	1/1	pyridine + CPI	1/20	pyridine	20	24	82	1.1/1	3.0	2.7	(2.0)	(1.7)

[a] Mole ratios. [b] Margin of error: ca. ±0.07 for values <2.0 and 0.1–0.15 for values >2.0. [c] 4-Chlorophenyl isocyanate (NCA/CPI = 80:1).

Table IV
Reaction Conditions and Results of the Copolymerization of Gly-NCA with L-Val-NCA

no.	Gly-NCA/ Val-NCA[a]	cat. + cocat.	solvent	temp, °C	time, h	yield, %	Gly/Val[b]	block length Gly	block length Val	reactiv ratios Gly	reactiv ratios Val
1	1/1	benzylamine	dioxane	20	24	31	4.5/1	8.0	1.7	7.0	0.7
2	1/1	triethylamine	dimethylacetamide	20	24	33	4.0/1	4.9	1.3	3.9	0.25
3	1/1	triethylamine + CPI[c]	dimethylacetamide	20	24	45	3.1/1	4.1	1.3	3.1	0.5
4	1/1	pyridine	pyridine	20	1	29	9.0/1	14.0	1.5	13.0	0.5
5	1/1	pyridine + CPI[c]	pyridine	20	1	20	12/1	17.0	1.4	16.0	0.4
6	1/1	pyridine + CPI[c]	pyridine	20	24	68	2.3/1	7.5	3.3	(6.5)	(2.3)
7	1/1			120	1	96	1.1/1	5.1	4.8	(4.1)	(3.8)

[a] Mole ratios. [b] Margin of error: ca. ±0.07 for values <2.0 and ca. 0.1–0.15 for values >2.0. [c] 4-Chlorophenyl isocyanate.

of incorporation not only depends on the reactivity of the NCA anions but also on their concentration. The concentration of these anions depends in turn on various parameters, e.g., N–H acidity of the monomers and the nature of the solvent.

All of these arguments demonstrate that the copolymerization parameters calculated from eq 3 do not have a simple kinetic significance as is true for the copolymerization of vinyl monomers. In the case of NCA copolymerizations, the r values have only a formal character and should be considered as "effective reactivity ratios". Nonetheless, these effective reactivity ratios provide worthwhile information for preparative purposes and allow one to study and to compare the influence of monomer structure and reaction conditions on the course of copolymerizations.

The above discussion demonstrates also that most problems with which an NMR spectroscopic investigation of NCA copolymerizations is confronted are inherent in the NCA polymerization and not in the spectroscopic analysis itself. Furthermore, we should like to emphasize that the ^{15}N NMR method possesses distinct advantages over the classical method: (A) One copolymerization experiment is in principle sufficient to determine the reactivity ratios. (B) The accuracy of the reactivity ratios is not affected by high conversions, if r_A and r_B are equal and the initial monomer concentrations are also equal. (C) When the copolymerization parameters are different (e.g., $r_A > 1$, $r_B < 1$), the error of the calculation according to eq 3 is systematic and its direction is predictable. With increasing conversion the higher value (r_A) becomes lower, while the lower parameter (r_B) becomes too high. This situation is demonstrated by experiments no. 7/8 and 9/10, in Table II, no. 1/2, 7/8, 9/10, and 11/13, in Table III, and no. 5/6, in Table IV. (D) Any individual sample of a copolypeptide can be characterized by the average length of the homogeneous blocks (eq 2). This characterization of the sequence is neither dependent on the conversion of a copolymerization nor does it require the knowledge of accurate copolymerization parameters. This aspect is important, because for many purposes high conversions are desirable, since both polymerization degree and yield increase with the conversion.

Thus, we come to the conclusion that this new method of sequence analysis represents an improvement compared with earlier methods, even if it is not highly accurate. Finally, it should be pointed out that this new kind of ^{15}N NMR and ^{13}C NMR sequence analysis can be successfully applied to many other copolymers such as copolyamides prepared from lactames[9,10] or copolyesters obtained from lactones.[11]

Results of the Copolymerization Experiments. In a previous paper we have reported that the copolymerization of Gly-NCA with Ala-NCA can be investigated by means of ^{13}C NMR spectroscopy,[12] while other copolypeptides by glycine are not amenable to a ^{13}C NMR sequence analysis. In this work γ-MeGlu-NCA and *S*-BzlCys-NCA were chosen as reaction partners for Gly-NCA because they were expected to possess a reactivity that is slightly lower than Ala-NCA but higher than Leu- and Val-NCA. Furthermore, both monomers possess heteroatoms in their side chains, while the side chains of both Leu-NCA and Val-NCA are free of hetereoatoms but branched. An interesting difference between the four reaction partners of Gly-NCA concerns the secondary structure of their polypeptides. The polymerization of Leu-NCA and γ-alkylGlu-NCA's leads to helical polypeptides while poly(*S*-BzlCys) prefers the pleated sheet structure, in analogy to polyvaline. Thus it was our intention to investigate the influence of the reaction conditions, as well as the influence of the secondary structure of the growing peptide chains, on the sequence of the copolypeptides.

The reaction conditions were selected with respect to the following mechanistic considerations. Primary amines are known to initiate the polymerization via a nucleophilic attack, and the propagation proceeds via analogous reaction steps involving an amino end group.[7] Secondary amines with relatively small substituents, such as diethylamine, are expected to behave analogously, while secondary amines with bulky substituents behave similarly to trialkylamines.[13-15] Triethylamine initiates the polymerization of N-unsubstituted NCA's by deprotonation[7,16] (eq 8) and the reaction of the resulting NCA anions with protonated monomers leads initially to a dimer (eq 9) which can continue its chain growth in two different ways. One possible propagation reaction is the so-called "activated monomer mechanism" (eq 10)[17-19] resulting from the nucleophilic attack of NCA anions onto the *N*-acyl-NCA end group of the growing chain. This mechanism is favored by polar solvents such as dimethylformamide, dimethylacetamide, or acetonitrile, because the equilibrium (eq 8) is shifted to the right. The "a.m. mechanism" is, furthermore, favored by the addition of electrophilic cocatalysts such as *N*-acyl-NCA's or isocyanates because then the formation of nucleophilic chain ends such as the carbamate or amino group is prevented.[7] In less polar solvents such as dioxane and in the absence of electrophilic cocatalysts, the propagation proceeds predominantly via carbamate chain ends (eq 11)[20] because the concentration of NCA anions is extremely low and because the initially formed *N*-acyl-NCA end groups disappear during the first stages of the polymerization. Experimental evidence for the instability of the *N*-acyl-NCA end groups and a discussion of the reactions leading to their dissappearance in the course of the polymerization were presented previously.[7,21] Compared with triethylamine, pyridine is a weak base but a good nucleophile. In high concentrations, pyridine can behave like a base, but nucleophilic attack leading to a zwitterion is also possible as demonstrated for several N-substituted NCA's and NTA's.[6] Since the formation of the zwitterions is followed chiefly by a propagation via the carbamate mechanism (eq 11), pyridine favors more than does triethylamine a chain growth via the carbamate mechanism. Experimental evidence for this assumption is presented by a ^{15}N NMR spectroscopic investigation of stereospecifity of the NCA polymerization.[22] Finally, it should be mentioned that a rapid polymerization of NCA is achievable by heating the pure monomers above 100 °C. Experiments no. 11, Table II, and no. 7, Table IV (Figure 1), demonstrate that under these conditions also copolypeptides are obtainable. However, the reaction mechanism of this "thermal polymerization" is unknown.

When the results of Table I are compared with those of Table II, the two following general conclusions can be drawn. First, γ-MeGlu-NCA is nearly as reactive as Gly-NCA, while *S*-BzlCys-NCA is slightly less reactive. The lower reactivity of *S*-BzlCys-NCA is obviously a consequence of the more bulky side chain. Second, the primary and secondary amine-initiated copolymerizations of Gly-NCA and γ-MeGlu-NCA show a weak tendency to produce block copolymers under all conditions except in experiment no. 6 (Table I). There may be two reasons why in many experiments both copolymerization parameters are found to be >1. First, one monomer is much more reactive than the other ($r_A > 1$; $r_B < 1$), but r_A and r_B are calculated from an experiment with high conversion. A copolypeptide from such a copolymerization possesses long blocks resulting from the less reactive monomer which must undergo homopolymerization, when the more reactive one is consumed. This situation is found when Gly-NCA is copolymerized with Leu-NCA or Val-NCA. However, in such cases r_B is sensitive to the conversion, as demonstrated by several experiments in Tables III and IV. The low or moderate conversion of experiments no. 4 and 5, in Table I, and the comparison of experiments no. 1 and 2, in Table I, indicate that in the case of γ-MeGlu-NCA r_A and r_B are >1 independent of the conversion. A similar preference for the formation of homogeneous blocks was not found in the experiments with *S*-BzyCys-NCA's and, thus, one may speculate that the formation of short helical blocks of Glu units is responsible for the predominant formation of homogeneous blocks in the experiments of Table I. However, the copolymerization of Gly-NCA with Ala-NCA[12] and with Leu-NCA (Table III) does not show an analogous tendency, although sequences of Ala and Leu units also prefer to adopt a helical secondary structure. Hence, a general relationship between the preferred secondary structure of polypeptides and the copolymerization parameters of the corresponding NCA's is not detectable at the current stage of our studies. When the experiments with diethylamine and diisopropylamine (no. 4 and 5, Table I, and no. 3, Table II) are compared with those carried out with primary amines or triethylamine, no exact agreement is found. This result is not surprising, since secondary amines can behave as nucleophiles (eq 7) and as bases (eq 8 and 9), so that the copolymerization parameter may be sensitive to the nature of the amine and to the nature of the NCA's. More interesting are the results obtained with triethylamine as catalyst. In the case of γ-MeGlu-NCA and Leu-NCA, the use of a polar solvent plus a cocatalyst (no. 8 and 9, Table I, no. 8, Table III) leads to pairs of copolymerization parameters which are rather different from those obtained in dioxane in the absence of a cocatalyst (no. 6, Table I, no. 3 and 4, Table III). Obviously the "a.m. mechanism" was operating in the former case while the "carbamate mechanism" was predominant under the latter conditions. As expected, these experiments carried out in polar solvent without cocatalyst or in dioxane with cocatalyst show an intermediate behavior (no. 7, Table I, no. 6 and 7, Table III). Surprisingly, the triethylamine-catalyzed experiments with *S*-BzlCys-NCA and Val-NCA do not show a similar sensitivity to the reaction conditions. However, all four series agree in that the addition of a cocatalyst to the triethylamine-catalyzed copolymerization in polar solvents leads to a remarkable acceleration of the polymerization. This result indicates that in all of these experiments (no. 8 and 9, Table I, no. 5 and 6, Table II, no. 8, Table III, and no. 3, Table IV) the "a.m. mechanism" was operating. This conclusion does not exclude the fact that other mechanisms, e.g., the carbamate mechanism (eq 11), have also contributed to the conversion, yet to a lesser extent than the "a.m. mechanism". Noteworthy with respect to preparative purposes is the observation that for both nearly ideal binary systems Gly/Glu and Gly/Cys, azeotropic copolymerizations are found when triethylamine is used as the catalyst in combination with dimethylformamide (no. 8 and 9, Table I, and no. 4–6, Table II). For the Gly/Leu and Gly/Val ideal systems, azeotropic copolymerizations were not found; however, also in these two cases the use of triethylamine in dimethylacetamide led to smaller differences between r_A and r_B than all other conditions. In other words, those conditions favoring the "a.m. mechanism" also favor reactivity ratios near 1. A further confirmation of this conclusion will be presented in a later part of this series for the copolymerization of Leu-NCA with Val-NCA. When pyridine is used as the catalyst, the results differ largely from those obtained with triethylamine, because the largest differences of r_A and r_B were here observed regardless of the comonomer used for Gly-NCA (no. 7, Table II, no. 11, Table III, no. 4 and 5, Table IV). Furthermore, it is noteworthy that the addition of 4-chlorophenyl isocyanate (CPI) does not have a distinct effect on the reactivity ratios and on the polymerization rate. Since pyridine is known to cause dimerization and trimerization of isocyanates, such side reactions of the cocatalyst are supposedly responsible for the above mentioned observation.

Finally, it should be pointed out that in the case of Gly/Leu and Gly/Val all copolymerizations reflect a large difference of reactivity between Gly-NCA and its comonomers. A lower reactivity of Leu-NCA and Val-NCA was expected, because their bulky side chains cause a steric hindrance to reactions of the nitrogen and of the carbonyl group C-5. The obvious consequence of largely differing reactivities is the formation of copolypeptides with block structure as demonstrated in Figures 5 and 6. Thus, it turns out that not only the reaction conditions but also the nature of the NCA's have a strong influence on the sequences of copolypeptides prepared from NCA's.

Conclusion

The present studies show that 36.48 MHz natural abundance ^{15}N NMR spectra allow one to investigate the copolymerization of Gly-NCA with other α-amino acid NCA's and to characterize the sequence of any individual copolypeptide obtained from these copolymerizations. The ^{15}N NMR sequence analysis is based on the fact that all four different kinds of peptide groups present in a binary copolypeptide show different signals. The ^{15}N NMR spectra of the four different copolypeptides investigated in this work display similar signal patterns, because the B-Gly bond resonates in all cases downfield of the Gly-Gly bond, while the Gly-B bond absorbs upfield of the B–B bond (Figures 3–6). These signal patterns agree with those of the glycine containing sequence polypeptides reported previously,[12] and they also agree with the results of our not yet published study on neighboring residue effects in oligopeptides. The quantitative evaluation of the ^{15}N NMR and ^{1}H NMR signals allows the calculation of average block lengths and of reactivity ratios. These parameters may be used to study the influence of the monomer structure and of the reaction conditions on the course of copolymerizations and on the sequence of the resulting copolypeptides.

The sequences of most copolypeptides obtained in this work and in a previous investigation on Gly-NCA/Ala-NCA[12] reflect the reactivities of the involved monomers: Gly = Ala ≥ γ-MeGlu > *S*-BzlCys ≫ Leu > Val. Different reactivities have the consequence that copolymers with a block structure are formed (e.g., the Gly/Val system), while equal reactivities favor the formation of random sequences (e.g., the Gly/Ala system). However, it must be emphasized that monomers with equal *r* values can also form block copolypeptides, if both *r* values are substantially greater than 1. Such a tendency—although weak—is observable for the system Gly-NCA/γ-MeGlu-NCA (Figure 3).

The above order of reactivities is based on the effective reactivitiy ratios of all experiments of a binary system initiated by primary and secondary amines or by pyridine.

Because the reactivities are averaged over experiments carried out under different conditions, the reactivity found in an individual experiment may deviate from this order. Strong base-initiated copolymerizations are principally not useful to establish an order of reactivities, because the rates of incorporation not only depend on the reactivity of the reactants but also on the concentration of NCA anions, on the stability of the *N*-acyl-NCA chain ends, and on which mechanism is predominant. An exact comparison of two binary systems requires, of course, the reaction conditions and the conversion to be identical. Anyway, it is conspicious that a comparison of the bulkiness of the NCA side chains leads to a similar order of aminoacyl units: Gly $<$ Ala $<$ γ-MeGlu $<$ *S*-BzlCys $\leq$ Leu $<$ Val. Thus we conclude that the steric effect of the side chains and the positive inductive effect of aliphatic substituents which lowers the electrophilicity of the NCA carbonyl group C-5 are the main factors that govern the effective reactivities.

Finally, it should be noticed that above all the triethylamine-initiated copolymerizations are sensitive to various parameters, e.g., polarity of the solvent or addition of cocatalysts. Obviously, these parameters have a strong influence on the reaction mechanisms in agreement with our previous observations.[22,23] Furthermore, it is noteworthy that the pyridine-initiated copolymerizations behave differently from the triethylamine-initiated ones but resemble the primary amine-initiated experiments. An analogous observation has been made in the case of the copolymerization of enantiomeric NCA's.[22] Thus, one can conclude that the chain growth of the pyridine-initiated polymerizations proceeds predominantly via nucleophilic chain ends and not by the activated monomer mechanism; yet this conclusion is tentative and deserves further confirmation. In this connection it should be mentioned that these results also agree with those obtained in our investigation of the copolymerization of Gly-NCA and β-Ala-NCA.[2] Whatever is the correct mechanistic interpretation, the results of the present and of our previous study[2] clearly demonstrate that the copolymerization of amino acid NCA's is influenced by various factors. The sequences of copolypeptides obtained from NCA's are, therefore, not always random as assumed by several authors investigating the properties of copolypeptides without characterization of the primary structure.

References and Notes

(1) Part 20. W. E. Hull and H. R. Kricheldorf, *Biopolymers*, in press.
(2) Unpublished results of W. E. Hull.
(3) (a) H. R. Kricheldorf, *Makromol. Chem.*, **180**, 147 (1979); (b) *Chem. Ber.*, **104**, 3146 (1971).
(4) F. R. Mayo and F. M. Lewis, *J. Am. Chem. Soc.*, **66**, 1594 (1944).
(5) J. Haarwood, *Angew. Chem.*, **77**, 405 (1965).
(6) H. R. Kricheldorf and K. Bösinger, *Makromol. Chem.*, **177**, 1243 (1976).
(7) H. R. Kricheldorf, *Makromol. Chem.*, **178**, 905 (1977).
(8) D. G. H. Ballard and C. H. Bamford, *Proc. R. Soc. London, Ser. A*, **223**, 495 (1954).
(9) H. R. Kricheldorf and W. E. Hull, *J. Polym. Sci., Polym. Chem. Ed.*, **16**, 2253 (1978).
(10) H. R. Kricheldorf and W. E. Hull, *J. Macromol. Sci.*, **11**, 2281 (1977).
(11) Study currently in progress.
(12) H. R. Kricheldorf and G. Schilling, *Makromol. Chem.*, **179**, 1175 (1978).
(13) M. Goodman and J. Hutchison, *J. Am. Chem. Soc.*, **88**, 3627 (1966).
(14) E. Peggion, M. Terbojevich, A. Cosani, and C. Colombini, *J. Am. Chem. Soc.*, **88**, 3630 (1966).
(15) H. R. Kricheldorf, *Makromol. Chem.*, **178**, 1959 (1977).
(16) H. R. Kricheldorf and M. Fehrle, *Chem. Ber.*, **107**, 3533 (1974).
(17) H. R. Kricheldorf, *J. Polym. Sci., Polym. Chem. Ed.*, **17**, 97 (1979).
(18) C. H. Bamford, H. Block, and A. C. Pugh, *J. Chem. Soc.*, 2057 (1961).
(19) M. Swarc, *Adv. Polym. Sci.-Fortschr. Hochpolym. Forsch.*, **4**, 1 (1965).
(20) R. Idelson and E. R. Blout, *J. Am. Chem. Soc.*, **79**, 3948 (1957).
(21) A. Cosani, G. D'Este, E. Peggion, and A. Scoffone, *Biopolymers*, **4**, 595 (1966).
(22) H. R. Kricheldorf and W. E. Hull, *Makromol. Chem.*, **180**, 1715 (1979).
(23) H. R. Kricheldorf, *Makromol. Chem.*, **178**, 905 (1977).
(24) H. R. Kricheldorf and W. E. Hull, *Makromol. Chem.*, **180**, 161 (1979).

Field Dependent Proton Spin–Lattice Relaxation Times for Characterization of Correlation Times in Dissolved Macromolecules

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ABSTRACT: The observation of proton spin–lattice relaxation times (T_1's) as a function of static magnetic field strength or Larmor frequency is considered as an approach to extend the dynamic range sensed in nuclear spin relaxation studies. Direct experimental measurements over a wide frequency range are needed because of the complicated character of local motions in chain molecules, and the utility of the proposed procedure is tested by conducting high resolution pulse Fourier transform proton T_1 studies on three dissolved polymers as a function of Larmor frequency over the range 20–90 MHz. For polyethylene dissolved in decalin, T_1 is nearly independent of Larmor frequency, while for both polycarbonate and poly(phenylene oxide) in $CDCl_3$, T_1 displays an appreciable dependence. In all three cases the field or frequency dependence can be accounted for in terms of a dynamic description based on motions likely in the respective polymers. The interpretations are also consistent with carbon-13 relaxation observed under the same conditions. Since both carbon-13 and proton data are acquired with a single spectrometer and yet the spectral density is probed from 20 to 180 MHz, greater confidence in the significance of dynamic model parameters is achieved relatively easily.

Models used to interpret nuclear spin relaxation in terms of local chain motions frequently involve several parameters.[1,2] These parameters correspond to a number of exponential correlation times reflecting motion over a fairly wide range of time scales. In order to determine model parameters with some certainty, thereby testing an interpretational model, an extensive set of spin relaxation measurements is required.[2] The most common ap-

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