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^{15}N-NMR SPECTROSCOPIC CHARACTERIZATION OF COPOLYAMIDES AND POLYPEPTIDES

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Abstract - ^{15}N-NMR signals of polyamides are sensitive to solvent and neighbouring residue effects. Hence, ^{15}N-NMR spectra are useful to distinguish blends of polyamides, alternating sequences and random copolyamides or to identify retroisomers. Furthermore, they allow the detection of transamidation reactions and the characterization of substituted polyamine-amides. ^{15}N-NMR spectra are inferior to ^{13}C-NMR spectra when a quantitative evaluation of copolyamide sequences is required; however, they are more useful for the sequence analysis of copolypeptides. ^{15}N-NMR signals are also highly sensitive to stereoisomerism, and thus, ^{15}N-NMR spectra can be used to analyze the tacticity of stereocopolypeptides (poly-D,L-amino acids).

Introduction

The sequence analysis of proteins is one of the oldest analytical problems of both biochemistry and macromolecule chemistry. It has been carried out by enzymatic cleavage of individual peptide bonds and by stepwise degradation from the amino end (Edman degradation). This kind of sequence analysis is nowadays automated by means of socalled sequenators; however, it requires a) amino end groups and b) all chains having identical sequences. Neither condition is fulfilled in the case of synthetic copolypeptides or poly-D,L-amino acids, so that spectroscopic methods are required to characterize their sequences.

Copolyamides resemble polypeptides in that chemical and enzymatic sequence analyses are not feasible. Since the different monomer units of copolyamides and polypeptides are linked by amide groups, it is obvious that the same spectroscopic methods should be applicable to both classes of polymers. Our first attempts to characterize copolyamide or polypeptide sequences by means of 220 MHz ^{1}H-NMR spectra were not successful except when the sequences were composed of 4-aminobenzoic acid and aliphatic ω-amino acids (1). ^{13}C-NMR spectroscopy turned out to be very useful for analyses of most copolyamide sequences (1-6); yet, it failed when applied to polypeptides, even when 90.5 MHz spectra are measured, except in the case of $(Ala/Gly)_n$ and $(Ala/Phe)_n$ (7). ^{13}C-NMR spectra were also found to be useless for sequences composed of various ß-amino acids and for tacticity analyses of poly-D,L-amino acids, except in the case of $(D,L\text{-}Leu)_n$, $(D,L\text{-}Val)_n$ and $(D,L\text{-}Ile)_n$ (8). These shortcomings of ^{13}C-NMR spectroscopy prompted us to test the potential use of ^{15}N-NMR spectroscopy for sequence analyses of copolyamides and polypeptides.

Copolyamides resulting from polymerizations and condensations

For spectroscopic reasons the copolyamides discussed in this work are divided into three classes:

I) Copolyamides containing aromatic amino acids or aromatic diamines and diacids.

II) Copolyamides made up of aliphatic ω-amino acids or aliphatic diamines and diacids.

III) Copolyamides resulting from chemical modification of polyamine-amides.

Copolyamides containing two different ω-amino acids or two different diamines and two diacids must contain four different kinds of amide groups:

a) the homogeneous bonds A-A and B-B and

b) the heterogeneous bonds A-B and B-A.

Sequence analyses are feasible if:

1) The signals of all four kinds of amide groups are wellresolved, so that a quantitative evaluation is possible.

2) At least three signals are sufficiently resolved (those of the A-B and B-A bonds overlapping).

3) All signals are unambiguously assigned.

Ternary copolyamides may contain nine different kinds of amide groups and resolution and assignments of at least seven signals is required for a successful sequence analysis.

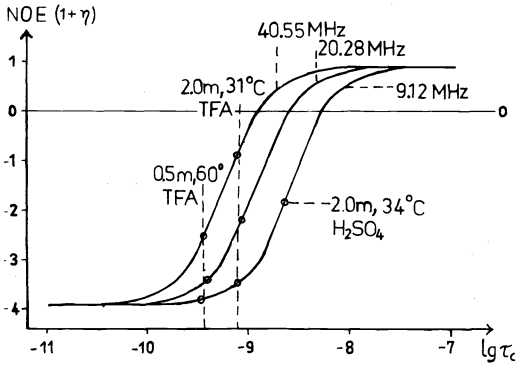


Fig. 1 Plot of calculated NOE values versus correlation time (τ) for three resonance frequencies and NOE measurements of various polyglycine ($\bar{P}_n$=50) solutions.

When ^{15}N-NMR spectra of random copolyamides of 4-aminobenzoic acid and 3-aminobenzoic acid or of 4-aminobenzoic acid and 6-aminohexanoic acid were measured in H_2SO_4 or FSO_3H solutions at 9.12 MHz and ca. 35°C, no signals were observed. This negative result was attributed to an unfavorable nuclear Overhauser effect (NOE). Fig. 1 demonstrates the dependence of the NOE on resonance frequency and correlation time τ (isotropic motion model), which is a measure of the segmental mobility of the dissolved polymers. As indicated in Fig. 1, nylon-2 (polyglycine) possesses NOEs in the range of -0.9 to -3.8, depending on solvent, concentration and temperature, when measured at 9.12, 20.28 and 40.55 MHz (9). Thus, it is expected that the stiffer chains of aromatic polyamides have NOEs near zero (signal nulling situation)

since they must be measured in viscous solvents (longer τ), such as H_2SO_4 or sulfonic acids. To avoid the problem of signal nulling, inverse-gated ^{1}H-decoupling measurements at 40.55 MHz were conducted. The broad signals (line-width 100-200 Hz) obtained in this way, did not show any fine structure suitable for sequence analysis. Hence, it may be stated that ^{13}C-NMR spectra are more useful for the sequence analyses of aromatic copolyamides because the carbonyl signals are sensitive to sequence effects (10) and have smaller line-width.

^{15}N-NMR spectra of aliphatic polyamides were found to be more promising. The line-widths of the homopolyamides were in the range of 6-15 Hz and dilute TFA solutions show a negative NOE even at 40.55 MHz. The homopolyamides were measured to reveal structure/shift relationships and to assign the homogeneous bonds (A-A, B-B) of copolyamides. A plot of the ^{15}N-NMR chemical shifts versus the chain length of ω-aminoacyl units resembles an exponential function demonstrating that all methylene groups in the main chain cause downfield shifts (11) (Table 1 and Fig. 2). Similar, but less pronounced chain length/shift functions were observed for polyamides made up of aliphatic α,ω-diamines and aliphatic or aromatic diacids (12) (Table 1). It is noteworthy that the shape of these shift functions depends strongly on the acidity of the solvent (11) (Fig. 2) because the chemical shifts of amide groups are highly sensitive to both hydrogen bonds and protonation.

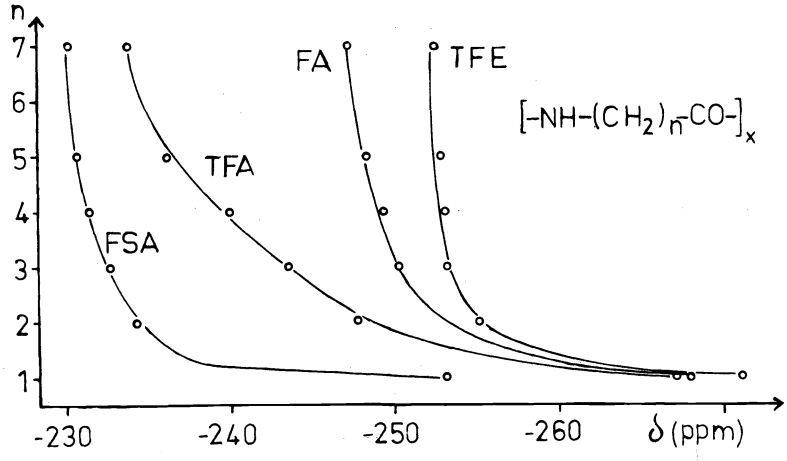


Fig. 2 ^{15}N-NMR chemical shifts (δ, upfield of external $NO_3^{\ominus}$) of various polyamides measured in fluorosulfonic acid (FSA), trifluoroacetic acid (TFA), formic acid (FA) and trifluoroethanol (TFE)

Copolyamides with alternating sequences of two different ω-amino acids were synthesized as models of the heterogeneous bonds (A-B; B-A) in random copolyamides. Their chemical shifts demonstrate that strong neighboring residue effects (NREs) exist in copolyamides, i.e. the chemical shift of a ω-amino acid unit under investigation (e.g. 6-amino hexanoic acid) depends on the nature of the comonomer (Table 1). Similarly strong NREs exist also in copoly-

amides containing ω-amino sulfonic acids (13). Hence, ^{15}N-NMR spectra allow one to distinguish alternating sequences from mixtures of the corresponding homopolymers or random copolyamides. In other words, ^{15}N-NMR allows one to check whether alternating copolyamides prepared by a new synthetic method, such as the condensation of the isothiocyanates of (1-3), give truely alternating sequences or random sequences due to transamidation. Similarly one can investigate whether a blend of homopolyamides undergoes fast transamidation in the molten state. When compared to ^{13}C-NMR spectra ^{15}N-NMR spectra have, of course, the shortcoming of a poor signal-to-noise ratio. However, the recently published application of cross-polarization (14) or INEPT (15) pulse techniques can improve substantially the ^{15}N-NMR signal intensities under suitable conditions. Furthermore, it is noteworthy that ^{15}N-NMR spectra allow the identification of retro-isomers of both polyamides and polypeptides (Fig. 3), (13, 16). Retro-isomers are built up by identical monomer units in such a way, that one sequence read from the amino to the carboxyl end matches the isomeric sequence read from the carboxyl to the amino end. This kind of sequence isomerism plays an important role for the understanding of relationships between primary structure, conformation and biochemical or pharmacological behavior of peptides (17).

$$n\ \text{SCN-A-CO-NH-B-COOH} \xrightarrow[-n\ \text{COS}]{} (\text{NH-A-CO-NH-B-CO-})_n$$

TABLE 1. ^{15}N-NMR chemical shifts δ (ppm, upfield of external $NO_3^{\ominus}$) of copolyamides made up of aliphatic and aromatic ω-amino acids

Polyamide	in TFA [a]	Polyamide	in TFA [b]
$(Gly)_n$	-266.2	$(Gly\text{-}3\text{-}Ala)_n$	-253.8; -261.7
$(3\text{-}Ala)_n$	-246.8	$(Gly\text{-}4\text{-}Ala)_n$	-252.0; -258.3
$(4\text{-}Abu)_n$	-242.6	$(Gly\text{-}5\text{-}Ava)_n$	-248.9; -258.9
$(5\text{-}Ava)_n$	-238.8	$(Gly\text{-}6\text{-}Aca)_n$	-247.7; -259.6
$(6\text{-}Aca)_n$	-235.1	$(3\text{-}Ala\text{-}4\text{-}Abu)_4$	-244.5; -246.1
$(D,L\text{-}3\text{-}Abu)_n$	-232.0	$(3\text{-}Ala\text{-}5\text{-}Ava)_n$	-241.7; -245.8
$(3\text{-}Abe\text{-}Gly)_n$	-243.); -266.1	$(3\text{-}Ala\text{-}6\text{-}Aca)_n$	-240.1; -145.4
$(3\text{-}Abe\text{-}3\text{-}Ala)_n$	-239.1; -250.9	$(4\text{-}Abu\text{-}5\text{-}Ava)_n$	-238.6; -245.4
$(3\text{-}Abe\text{-}4\text{-}Abu)_n$	-238.4; -249.1	$(4\text{-}Abu\text{-}6\text{-}Aca)_n$	-236.8; -244.3
$(3\text{-}Abe\text{-}5\text{-}Ava)_n$	-239.1; -243.5	$(5\text{-}Ava\text{-}6\text{-}Aca)_n$	-236.6; -237.9
$(3\text{-}Abe\text{-}6\text{-}Aca)_n$	-239.7; -241.6	$(6\text{-}Aca\text{-}3\text{-}Abu)_n$	-231.3; -239.4
$(3\text{-}Abe\text{-}3\text{-}Abu)_n$	-238.5; -237.3	$(6\text{-}Aca\text{-}Gly\text{-}Gly)_n$	-247.2; -259.4; -266.7
$(4\text{-}Abe\text{-}3\text{-}Ala)_n$	-238.4; -251.1	$(6\text{-}Aca\text{-}3\text{-}Ala\text{-}Gly)_n$	-242.1; -248.6; -261.9
$(4\text{-}Abe\text{-}6\text{-}Aca)_n$	-238.8; -241.7	$(6\text{-}Aca\text{-}Gly\text{-}3\text{-}Ala)_n$	-236.9; -254.5; -260.5

a) Abe = aminobenzoic acid

b) The order of δ-values matches that of monomer units in the formulas

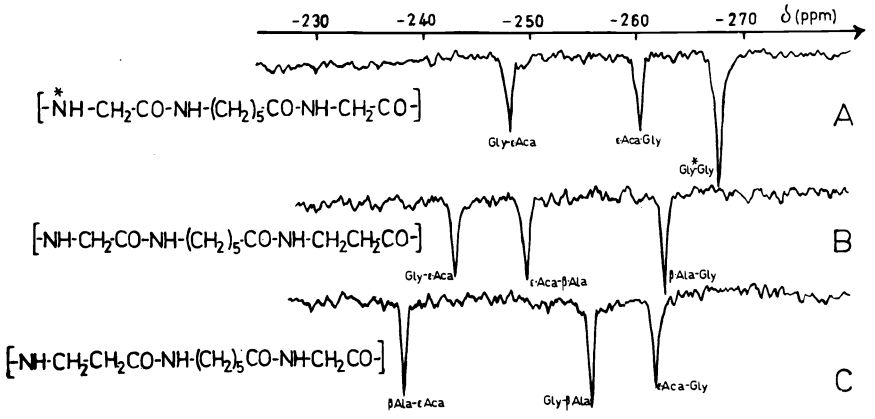


Fig. 3 9.12 MHz ^{15}N-NMR spectra of the sequence polyamides (Gly-6-Aca-Gly)$_n$ (A); (Gly-6-Aca-3-Ala)$_n$ (B) and (3-Ala-6-Aca-Gly)$_n$ (C) measured in TFA at 30°C

A disappointing result was obtained upon investigation of random copolyamides resulting either from anionic copolymerizations of lactams, e.g. nylon-4/-6; nylon-4/-8 and nylon-6/-12, or from condensation of ω-amino acids, diamines and diacids (e.g. nylon-6,6/-6). The signals appeared at those positions predicted from the homopolyamides and from the alternating sequences; yet, in all cases the linewidth was unexpectedly great (30-60 Hz) and both, resolution and signal-to-noise ratio were poor. Thus, in neither case were we able to calculate the average lengths of the homogeneous blocks from the ^{15}N-NMR spectra (even when measured at 40.55 MHz). The ^{13}C-NMR spectra gave significantly better results (3-6). The main reason for the unfavorable line-widths are unresolved long range NREs, i.e. NREs of monomer units not directly linked to the nitrogen under investigation. These long range NREs may reach the order of 2-3 ppm as demonstrated in Fig. 3 for the Gly-Aca bond in Gly-6-Aca-Gly)$_n$ and (Gly-6-Aca-3-Ala)$_n$ or for the Aca-Gly bond in (Gly-6-Aca-Gly)$_n$ and (Aca-Gly-3-Ala)$_n$. These long range NREs are relatively strong when ω-amino acids (or diamines) of different chain length are combined in a sequence. They are relatively weak, when the monomer units differ only by their side chains. Thus. wellresolved signal patterns with line-widths in the range of 15-20 Hz were found, when copolyamides of ß-alanine and 3-aminobutyric acid or ß-alanine and 3-amino-isobutyric acid were investigated. The former copolyamides were obtained by hydrogen-transfer polymerizations of acrylamide and methacrylamide (18). As Fig. 4 demonstrates the sequences are between random and blocky, because acrylamide is more reactive than methacrylamide. In contrast ß-alanine-N-carboxy anhydride (ß-Ala-NCA) 2a and 3-aminoisobutyric acid-NCA 2b (19) possess equal reactivities and the resulting copolymers are truely random. Our observation that long range NREs are weak and that the ^{15}N-NMR spectra of copolymers are wellresolved when the monomer units differ only by their substituents holds also for polypeptides. Table 2 summarizes the relationships between monomer structure, NREs and usefulness of ^{13}C- and ^{15}N-NMR sequence analysis. It is obvious, that both nuclei complement one another.

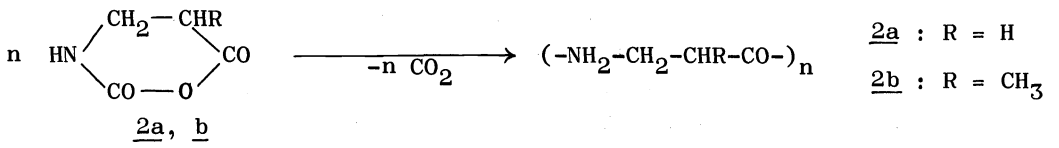


TABLE 2. Strengths of neighboring residue effects. Consequences for the NMR spectroscopic sequence analyses of copolyamides and copolypeptides

Comonomers	^{15}N-NMR Spectra	^{13}C-NMR Spectra
1) ω-amino acids with varying distance between amino- and carboxyl group	strong direct NREs strong long range NREs broad signals poor resolution	strong direct NREs weak long range NREs sharp signals good resolution
2) α- and ω-amino acids with varying side chain	strong direct NREs weak long range NREs sharp signals good resolution	weak direct NREs strong long range NREs broad signals poor resolution

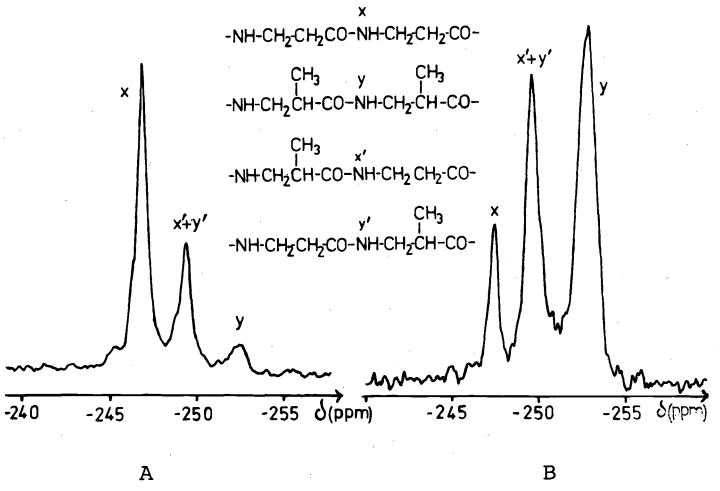


Fig. 4 40.55 MHz ^{15}N-NMR spectra (measured in TFA) of copolyamides obtained by hydrogen-transfer copolymerization of acrylamide and methacrylamide A.) initial monomer ratio 1:1 B) same reaction conditions, but monomer ratio 1:4

Modification of Polyamides

In addition to copolymerization, cocondensation and transamidation chemical modification of existing polyamides is the fourth route to produce copolyamides. Although a direct substitution of amide groups is feasible (20, 21), pol amides with additional functional groups are more suitable for chemical modifications. The technically produced polyamine amide 3 seems to be a cheap and useful starting material for a variety of reactions and applications.

Alkylated and acylated derivatives may be useful as drug carriers, ion complexing reagents, membranes or ion exchange resins (after crosslinking). Because polyamide 3 is supplied in the form of a concentrated water solution and because any potential application requires conversion in water, ^{13}C- and ^{15}N-NMR spectroscopy are more useful than ^{1}H-NMR spectroscopy to follow the course of the reaction.

$$\left[-CO-(CH_2)_4-CO-NH-CH_2-CH_2-NH-CH_2-CH_2-NH-\right]_n \quad 3$$

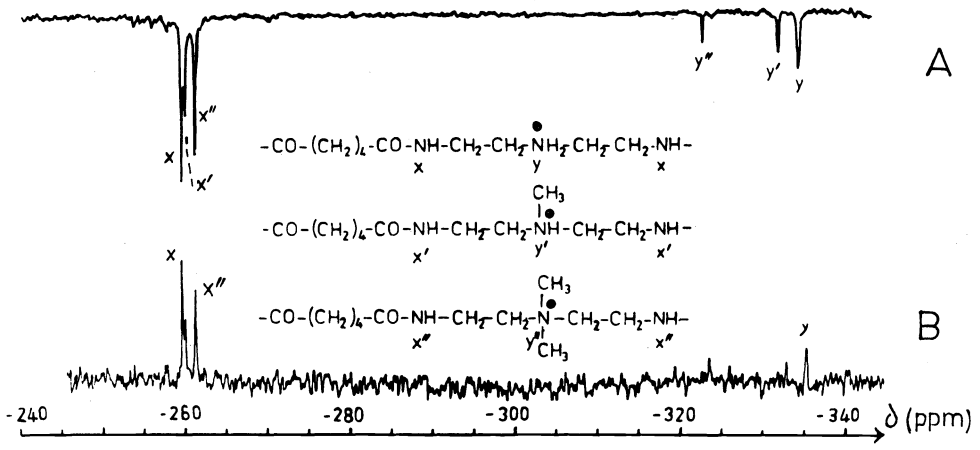


Fig. 5 20.28 MHz ^{15}N-NMR spectrum of the reaction product (in H_2O) resulting from the alkylation of polyamine amide 3 with methyl methanesulfonate in water at 0°C: A) continuous broad band ^{1}H-decoupling; 19.500 transients B) inverse-gated ^{1}H-decoupling; 7.000 transients

Alkylation with methyl methanesulfonate, leading to a cationic polyelectrolyte, and acylation with succinic acid anhydride, leading to an anionic polymer, are two examples which will be discussed here in more detail (18). The first step of the methylation is the formation of tertiary amino groups along with free methane sulfonic acid. Since secondary amino groups are more basic in water than tertiary ones, the sulfonic acid protonates predominantly the secondary amino groups and the methylation of NH-groups stops after ca. 60% conversion. The free tertiary amino groups react partially with unreacted methyl methane sulfonate yielding quarternary ammonium groups. Correspondingly, the ^{15}N-NMR spectrum exhibits signals of three different ammonium groups (Fig. 5 A). However, due to different NOEs and relaxation times (T_1) a quantitative evaluation of their intensities is not feasible. Interestingly, the ammonium groups exert strong upfield shifts on the neighbored amide groups. These shielding effects are attributed to an electric field effect (22) of the ammonium groups, since inductive effects cause downfield shifts. The amide signals possess nearly identical NOEs and relaxation times and are, thus, useful for a quantitative evaluation (Figs. 5 A/B). In this way, the molar ratio of sec.-, tert.- and quart.- ammonium groups was found to be 40 : 25 : 35. The acylation of 3 with succinic acid anhydride at 0°C stops as expected after ca. 55% conver-

sion, because the free carboxyl groups protonate unreacted sec. amino groups. Addition of NaOH and excess succinic anhydride leads to more than 90% conversion (18). The spectroscopic consequence of this acylation is

a) formation of sec. ammonium groups combined with an upfield shift of the neighboring CO-NH groups (signal x in Fig. 6);
b) appearance of the succine amide signal which is downfield shifted due to the combined ß-effects (23) of the two ethylene groups;
c) signal splitting of the amide groups neighboring the succinamide units. This splitting reflects the cis/trans isomerism of the succinamide group (Fig. 6).

Both examples, the alkylation and the acylation of polyamine amide 3, demonstrate how useful the sensitivity of ^{15}N-NMR signals to long range NREs may be for the characterization of polyamide structures.

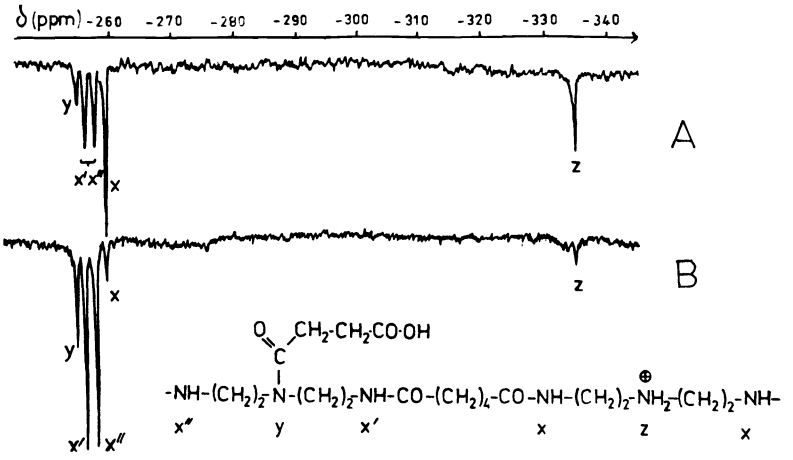
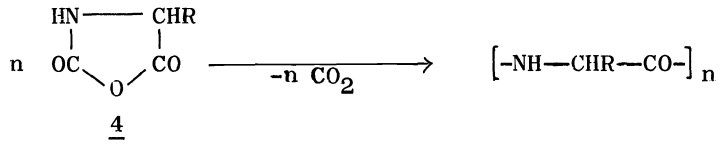


Fig. 6 9.12 MHz ^{15}N-NMR spectra of polyamine amide
A) acylated with 50 mol% succinic anhydride in water at 0°C
B) acylated with an equimolar amount of succinic anhydride and 4 N NaOH in water at 0°C

Copolypeptides

In the past three decades copolypeptides and homopolypeptides were studied as models of proteins. While the composition of the copolypeptides was easily measurable by amino acid analysis and ^{1}H-NMR spectra, sequence analyses were not feasible. Hence, copolypeptides were usually considered to possess random sequences. In the case of glycine/alanine copolymers Oya et al. postulated an almost alternating sequence (24); yet both, ^{13}C-NMR spectra (7) and ^{15}N-NMR spectra (25), clearly show that random sequences are formed upon primary amine-initiated copolymerization of glycine-NCA and alanine-NCA. There are two reasons why detailed and unambiguous sequence information of copolypeptides is highly desirable. First, copolypeptides are in most cases obtained by copolymerization of α-amino acid NCAs (4). Various reagents can serve as initiators; the polymerization mechanisms are complex and not yet fully elucidated. Since the sequence of a copolymer reflects the kinetic course of the copolymerization, sequence analyses of copolypeptides prepared under various

reaction conditions may contribute to the elucidation of the polymerization mechanism. Second, the properties of copolypeptides depend on their sequences. This aspect is of particular importance for investigation of the helix stability. If, for instance a helix-forming and a helix-destabilizing monomer are copolymerized, the degree of helicity will be higher for a block copolymer than for a random sequence.



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The feasibility of ^{15}N-NMR sequence analyses was first detected when sequence polypeptides of the type $(X\text{-}Gly\text{-}^{*}Gly)_n$ with X=3-Ala, Phe, Pro, Val etc. (the asterisk denotes ^{15}N-enrichment) were measured (26, 27). The two glycine nitrogens showed different chemical shifts, and the shift difference was dependent on the nature of X-indicating strong direct NREs. Since the X-Gly and Gly-X bonds of the sequence polypeptides are models of the A-B and B-A bonds, their chemical shifts and those of homopolypeptides (models of A-A and B-B bonds) allow unambiguous assignments of all signals of the corresponding copolypeptides. In this way, the signal patterns of glycine-containing copolypeptides were analyzed (28, 29). The chemical shifts of numerous homogeneous and heterogeneous peptide groups measured in TFA are listed in Table 3. Because it is time-consuming and expensive to synthesize partially ^{15}N-enriched sequence polypeptides, a second method was developed to assign the A-B and B-A bonds of other copolypeptides. For this purpose 50% ^{15}N-enriched amino acid NCAs were copolymerized with the 50-fold excess of other amino acid NCAs containing the natural abundance of ^{15}N. The resulting copolypeptides contain individual ^{15}N-enriched A-monomers flanked by blocks of B-monomers (so-called guest-host copolypeptides (25)). Hence, the chemical shifts of the ^{15}N-enriched guest-monomers (A) represent the B-A bonds in copolypeptides.

TABLE 3. ^{15}N-NMR chemical shifts δ (ppm, upfield of external $NO_3^{\ominus}$) of peptide groups in polypeptides dissolved in TFA

Peptide	δ a)	Peptide	δ a)	Peptide	δ a)
Gly-Gly	-266.1	Ala-Gly	-266.8	Gly-Ala	-250.2
Ala-Ala	-250.6	Val-Gly	-262.9	Gly-Val	-254.3
Val-Val	-248.7	Leu-Gly	-265.0	Gly-Leu	-251.9
Leu-Leu	-249.5	Phe-Gly	-263.6	Gly-Phe	-253.7
Phe-Phe	-250.4	Glu-Gly	-265.0	Gly-Glu	-254.6
Glu-Glu b)	-252.8	Val-Ala	-246.2	Ala-Val	-254.8
Met-Met	-251.9	Leu-Ala	-248.5	Ala-Leu	-251.5
		Glu-Ala	-248.3	Met-Glu	-254.6
		Phe-Ala	-247.2	Ala-Phe	-253.3
		Leu-Val	-251.3	Val-Leu	-247.2
		Glu-Val	-250.3	Val-Glu	-249.1
		Glu-Leu	-250.3	Met-Ala	-248.3

a) margin of error $\pm$ 0.1 ppm b) γ-Methyl glutamate

A first series of copolymerization was condected with glycine-NCA, on the one hand, and alanine-NCA, γ-methyl glutamate-NCA, leucine-NCA, S -benzylcysteine-NCA and valine-NCA, on the other hand. In all five cases the characteristic pattern of four signals was wellresolved, when measured at 40.55 MHz (29). Inverse-gated ^{1}H-decoupled spectra allowed the quantitative evaluation of signal intensities yielding the following results. First, nearly random sequences are obtained when the comonomers of glycine-NCA possess unbranched side chains (Ala, Glu, Cys). Second, leucine-NCA and valine-NCA polymerize slower than glycine-NCA due to their bulky side-chains, and at high conversion copolypeptides with a blocky structure are obtained. However, when two less reactive monomers, such as leucine-NCA and valine-NCA, are copolymerized, again random sequences are found (30). Third, the reaction conditions do not have a strong influence on the primary structure of copolypeptides, except when glycine-NCA is copolymerized with ß-alanine-NCA (28). In favorable cases, such as the terpolymerization of Gly-NCA, Leu-NCA and Val-NCA, sequences built up by three different monomer units can be analyzed (30). Again, model polypeptides provided the basis for the assignments of all nine different kinds of peptide groups because oligopeptides, which are easier to synthesize, are not reliable models (31). The ^{15}N-NMR spectra along with some kinetic data also allow us to characterize the chemical heterogeneity of second order, i. e. variation of the sequence along the peptide chain. For example, the terpolypeptide of Fig. 7 resulting from a diethylamine-initiated NCA polymerization may be approximated by the formula $H\text{-(Leu/Val)}_x\text{-(Gly/Leu/Val)}_y\text{-(-Gly)}_2\text{-NEt}_2$ (30). Finally, it is noteworthy that the sequences are best characterized by calculation of the average lengths of the homogeneous blocks (I_A and I_B) according to the following equations (I_{AA} = signal intensity of A-A bonds etc.):

$$\bar{L}_A = \frac{I_{AA}}{I_{AB}} + 1 \quad \text{(copolypeptides)}; \qquad \bar{L}_A = \frac{I_{AA}}{I_{AB} + I_{AC}} + 1 \quad \text{(terpolypeptides)}$$

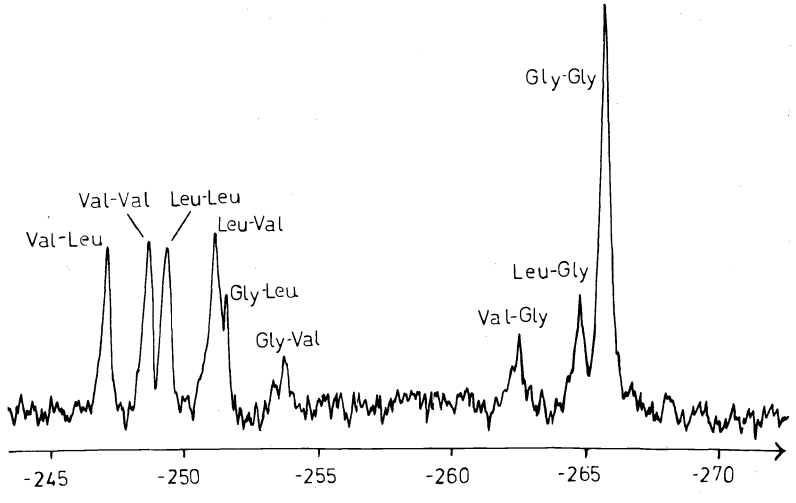

Fig. 7 40.55 MHz inverse-gated ^{1}H-decoupled ^{15}N-NMR spectrum of $Et_2N(Gly/Leu/Val)_n$ in TFA

Sequence and secondary structure

Numerous investigations based on X-ray, IR, CD and ORD spectroscopy have shown that poly-L-alanine adoptes preferentially the α-helix structure in both the solid state and in solution. In contrast, glycine and valine are known to be helix destabilizing units, because the homopolymers prefer the pleated sheet structure. Hence, incorporation of glycine or valine units into poly-alanine is expected to destabilize the α-helix more or less depending on their concentration and distribution in the chain. By means of ^{13}C- and ^{15}N-NMR spectroscopy we were able to demonstrate (32, 33) that solutions of poly-L-alanine in pure TFA contain helical and a non-helical conformation. The equilibrium between both secondary structures is sensitive to various parameters such as temperature, chain-length,and content of helix-destabilizing comonomers. Three series of copolymers were synthesized to study the influence of helix-destabilizing amino acids on the helix-coil equilibrium of poly-L-alanine in TFA:

a) $(L\text{-}Ala)_{100}$ containing 2, 5, 10 and 20 mol% D-Ala-units, obtained by benzylamine-initiated NCA copolymerization at 100°C in dioxane.
b) $(L\text{-}Ala)_{100}$ containing 2, 5, 10 and 20% Gly- units, obtained by benzylamine-initiated NCA copolymerization in dioxane at 100°C.
c) $(L\text{-}Ala)_{100}$ containing 5, 10 and 20% L-Val units, obtained by benzylamine-initiated NCA copolymerization in dioxane at 20°C.

^{15}N-NMR spectroscopy sequence analyses have shown that both copolymerizations, L-Ala-NCA/D-Ala-NCA and L-Ala-NCA/Gly-NCA in dioxane at 100°C lead to nearly random sequences (7, 34). Accordingly, it was found that both guest units have a strong influence on the helix coil equilibrium of $(L\text{-}Ala)_{100}$, so that with increasing content of Gly or D-Ala units the concentration of helices decreased. This effect was much less pronounced with L-valine as comonomer in agreement with the fact that the copolymerization of L-Ala-NCA and L-Val-NCA leads to block copolymers at 20°C in dioxane (33). It is, thus, obvious that the helix stability of copolypeptides depends significantly on their sequences.

Furthermore, 30.5 MHz ^{15}N-NMR spectra of solid guest-host copolypeptides were measured using the cross-polyrization/magic-angle-spinning technique (35). ^{15}N-enriched poly-L-leucine ($\bar{P}_n$ = 50), prepared by benzylamine-initiated NCA polymerization in dioxane at 20°C, was studied first. This sample exhibited two signals, a weak one at -246 ppm (upfield of solid $NH_4{}^{15}NO_3$) resulting from the pleated sheet structure and a strong one of the α-helices at -258 ppm (Fig. 8 A). The simultaneous presence of both secondary structures agrees well with the results of X-ray and IR-studies of japanese authors (36, 37). When ^{15}N-enriched L-Leu units were incorporated into poly-L-valine, which cannot adopt the α-helix conformation, only the signal at -246 ppm was observable, indicating that the L-Leu units participate in the pleated sheet structure (Fig. 8 B). When ^{15}N-enriched L-valine was incorporated into a poly-leucine it absorbed at -257 ppm a value characteristic for a helical environment (Fig. 8 C). These observations along with those of other guest-host copolypeptides clearly demonstrate that the chemical shifts of solid polypeptides are dominated by their secondary structure, while NREs are negligible (35). The shift

differences of dissolved coil and helices are only in the range of 1.0 - 1.2 ppm (33, 38), while NREs may reach 10 ppm. Thus, solid and dissolved copolypeptides show an opposite spectroscopic behavior.

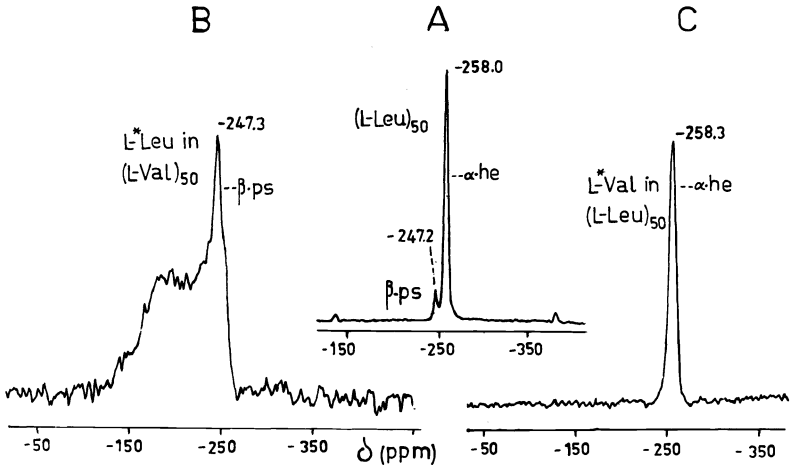

Fig. 8 30.42 MHz ^{15}N-NMR CP/MAS spectra A) poly-L-leucine containing 10% ^{15}N; B) poly-L-valine containing 2 mol% ^{15}N enriched L-Leu units; C) poly-L-leucine containing 2 Mol% ^{15}N enriched L-Val units

Tacticity of Poly-D,L-amino acids

Since the first report of Lundberg and Doty on the kinetic features of the polymerization of D,L-amino acid NCAs (39), the stereospecificity of D,L-NCA polymerizations has attracted much interest. It has been concluded by several authors (40) that the polymerization of D,L-amino acid NCAs is highly stereospecific, favoring exclusively the formation of isotactic blocks (L-L or D-D sequences), and the helical secondary structure of the growing peptide chain was considered to be responsible for the steric course of the polymerization. However, all previous investigation of this problem used rather indirect methods, such as CO_2-kinetics, optical rotation measurements and enzymatic degradation. Only recently it has been demonstrated that highresolution ^{13}C- and ^{15}N-NMR spectra of poly-D,L-amino acids display signal patterns with tacticity splittings which allow a direct investigation of the stereosequences and of the stereospecificity of polymerizations of D,L-α-amino acid NCAs. While the ^{13}C-NMR method is limited to poly-D,L-amino acids with branched side-chains, the smallest side-chain, i. e. the CH_3 group of $(D,L\text{-Ala})_n$, suffices to cause tacticity splittings of the ^{15}N-NMR signal (34).

Tacticities of poly-D,L-amino acids may be described by diads, triads, tetrads etc. in analogy with tacticities of vinyl polymers (Table 4). However, one characteristic difference is noteworthy, namely the directional sense of the peptide chain, which implies that four instead of three triads can exist and eight tetrads instead of six. The ^{15}N-NMR spectroscopic tacticity analysis of poly-D,L-amino acids is confronted with three difficulties:

I) Low signal-to-noise ratio, because of the broad signals (line-width of the entire signal 2-6 ppm).
II) Insufficient resolution of diad, triad or tetrad peaks.
III) Assignment of the individual diad, triad and tetrad peaks.

TABLE 4 L-centred stereosequences of poly-D,L-amino acids (the enantiomeric D-centered stereosequences are not shown)

Diads:	L-$_N$-L	(i)	L-$_N$-D	(s)
Triads:	L-$_N$-L-L	(ii)	L-$_N$-D-L	(ss)
	L-$_N$-L-D	(is)	L-$_N$-D-D	(si)
Tetrads:	L-L-$_N$-L-L	(iii)	L-L-$_N$-D-L	(iss)
	D-L-$_N$-L-L	(sii)	D-L-$_N$-D-L	(sss)
	L-L-$_N$-L-D	(iis)	L-L-$_N$-D-D	(isi)
	D-L-$_N$-L-D	(sis)	D-L-$_N$-D-D	(ssi)

A signal-to-noise ratio sufficient for a quantitative evaluation of the signal patterns can be achieved in two ways. First, by ^{15}N enrichment $\geqq$ 2% and second, by use of the INEPT pulse technique (15). The resolution depends on the nature of the poly-D,L-amino acid under investigation. However, the solvent has the greatest influence. For instance, a broad signal withoug any fine structure is obtained, when $(D,L\text{-Leu})_n$ is measured in pyridine, while a wellresolved signal pattern exhibiting even tetrad peaks is observable in TFA. Further increase of the solvent acidity by addition of methane sulfonic acid leads to decreasing resolution (25). Assignments of isotactic (i) and syndiotactic (s) diads and triads were achieved by comparison with the model polymers 5 and 6 which resemble the above discussed guest-host copolypeptides (* denotes 50% ^{15}N-enrichment):

$$-(D\text{-}A)_n\text{-}L\text{-}\overset{*}{A}\text{-}(D\text{-}A)_m- \qquad \underline{5}$$

$$-(D\text{-}B)_n\text{-}L\text{-}\overset{*}{A}\text{-}(D\text{-}B)_m- \qquad \underline{6}$$

$$-(-L\text{-}A\text{-}D\text{-}A\text{-}L\text{-}\overset{*}{A}\text{-})_x- \qquad \underline{7}$$

The model polypeptides 6 are designed to allow assignments of tacticity peaks of socalled hetero-stereocopolypeptides, i.e. copolypeptides containing the L-form of one amino acid and the D-form of another one. The measurements of numerous stereomodel polypeptides revealed the following stereoscopic rule (25):

"Isotactic diads, triads or tetrads absorb downfield of the corresponding syndiotactic diads, triads or tetrads"

This rule holds also for most oligopeptides (41) and cyclopeptides (42). Thus, it may be useful for identifying various kinds of diastereomeric peptides. Heterotactic triads, (is) and (si), have not yet been assigned. For this pur-

pose the stereosequence polypeptides 7 are required; their syntheses are in progress.

The results obtained from ^{15}N-NMR tacticity analyses of $(D,L\text{-Ala})_n$, $(D,L\text{-Val})_n$; $(D,L\text{-Leu})_n$; $(\gamma\text{-OMe},D,L\text{-Glu})_n$ and $(D,L\text{-Phe})_n$ may be summarized as follows:

A) The stereospecificity of all polymerizations is low; the average length of isotactic (or syndiotactic) blocks is ≤ 5.

B) Under most reaction conditions the formation of isotactic blocks is favored (Fig. 9 A); yet a preferred formation of syndiotactic sequences (ss) may accur (Fig. 9 B).

C) The nature of the secondary structure does not have a greater influence on the stereospecificities then have other parameters, such as solvent, temperature etc.

D) From the intensities of triad and tetrad peaks it may be concluded that the second to last and even the third to last monomer unit of the active chain-end affects the stereospecificity.

Finally, it is worth mentioning that not only stereospecificities of polymerizations but also stereospecificities of any kind of peptide syntheses as well as racemizations may be elucidated by means of ^{15}N-NMR spectra (34, 43, 44).

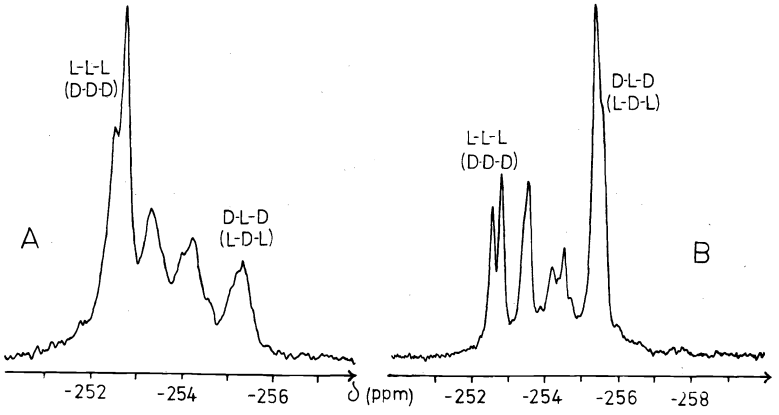

Fig. 9 40.55 MHz inverse-gated ^{1}H-decoupled ^{15}N-NMR spectra of $(\gamma\text{-OMe-D,L-Glu})_n$ obtained A) in dioxane at 20°C with benzylamine as initiator, B) in pyridine/benzene (1:2 by volume) at 20°C

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