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^{15}N-NMR Spectroscopy. V. Investigation of Peptides and Sequence Polyamides Containing ω-Aminosulfonic Acids

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^{15}N-NMR Spectroscopy. V. Investigation of Peptides and Sequence Polyamides Containing ω-Aminosulfonic Acids

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ABSTRACT

Natural abundance ^{15}N-NMR spectra of ten different sequence polymers containing taurine, γ-aminopropanesulfonic acid, or sulfanilic acid as well as various aminocarboxylic acids were measured in trifluoroacetic acid. Strong neighboring residue effects and a relationship between chemical shift and chain length of the ω-amino acyl residues are observed and discussed. Isomeric sequences of first and second order like (Tau-γ-Abu)$_n$ and (γ-Aps-β-Ala)$_n$ or (Tau-Gly-β-Ala)$_n$ and (Tau-β-Ala-Gly)$_n$ show different spectra. The deprotonation of the sulfonamide groups in alkaline solution results in a downfield shift as well as a substantial decrease in signal intensity.

INTRODUCTION

In previous papers we have demonstrated that ^{13}C-NMR spectroscopy is a useful analytical tool for the sequence analysis of copolypeptides and copolyamides [1-6]. Copolyesters and polyesteramides can likewise be characterized by ^{13}C-NMR spectra [7, 8]. This characterization is based on the fact that the carbonyl groups are sensitive to neighboring residue (or sequence) effects. Since aminosulfonic acids do not possess carbonyl groups, polymers containing such monomer units are less prone to a characterization by ^{13}C-NMR spectroscopy. On the other hand, the results of our preliminary investigations on ^{15}N-NMR spectra of polypeptides indicate that, in general, ^{15}N-NMR spectroscopy is more suitable for the sequence analysis of copolymers than ^{13}C-NMR spectroscopy [9-11]. Since in polypeptides containing ω-aminosulfonyl residues all monomer units possess a nitrogen atom, ^{15}N-NMR spectroscopy should be the appropriate method for a spectroscopic characterization of such copolymers.

In a recent paper we described for the first time the synthesis of sequence polypeptides containing ω-aminosulfonic acids [12]. Since a new class of copolymers was prepared by a new condensation method, it was necessary to confirm that alternating sequences were really obtained. For this purpose a spectroscopic method is advantageous over an analytical method based on thermal, chemical, or enzymatic degradation, because it is nondestructive and time-saving. Moreover, sequence polymers are useful models for studying relationships between primary structure of copolymers and ^{15}N-NMR chemical shifts. In this connection it should be pointed out that, so far as we know [13], no ^{15}N-NMR data on sulfonamides have been reported. Thus, beyond the special purpose of sequence analysis, this paper should provide some basic information on the spectroscopic behavior of sulfonamide groups.

EXPERIMENTAL

9.12 MHz ^{15}N-NMR Spectra

These spectra were measured on a Bruker WH-90 FT-NMR spectrometer at a magnetic field length of 21 KGauss. A pulse width of 60 μsec (ca. 70-80°) was used, and ca. 40000 scans were accumulated with 2K data points on a spectral width of 500 Hz in the case of the polymers or with 4K data points on 1000 Hz in the case of tripeptides. Spectra of 1.3 g portions of polymer dissolved in 7 ml trifluoroacetic acid were measured in 20 mm diameter sample

tubes at 32-35°C. A coaxial capillary (5 mm diameter) containing D_2O served as a lock. After each measurement this capillary was replaced by another one containing an isotopically enriched $NH_4^{\oplus}NO_3^{\ominus}$ solution in D_2O. The $NO_3^{\ominus}$ ion served as standard for both chemical shift and phase correction. The resonance frequency of the $NO_3^{\ominus}$ ion turned out to be insensitive to the nature of the solute and its concentration. The nuclear Overhauser effect was strongly negative in all measurements.

18.24 MHz ^{15}N-NMR Spectra

These spectra were measured on a Bruker WH-180 FT-NMR spectrometer equipped for quadrature detection at a magnetic field strength of 42 KGauss. A pulse width of 20 μsec (ca. 30°) was used, and ca. 20000-30000 scans were accumulated with 4K data points on a spectral width of 3000 Hz. Spectra were measured on 3 g portions of polymer dissolved in 20 ml solvent at 30-35°C in 25 mm diameter sample tubes with a 5 mm diameter coaxial capillary.

RESULTS AND DISCUSSION

Alternating Binary Copolyamides

The synthesis of the binary copolyamides Ia-d, IIa and IIb, and III was described in a recent paper [12]. Their spectra were measured in trifluoroacetic acid, because this solvent allows comparisons with many other polypeptides or polyamides and because its viscosity is extremely low (Table 1).

$$\cdots[-NH-CH_2-CH_2-SO_2-NH-(CH_2)_m-CO-]_n\cdots$$

I

a: m = 1
b: m = 2
c: m = 3
d: m = 5

If the tauroyl polymers Ia-d are compared with the corresponding homopolyamides nylon 2 to nylon 6, analogous behavior is found. Increasing chain length of the aminoacyl residues results in a distinct

TABLE 1. ^{15}N-NMR Chemical Shifts δ (Relative to External $NO_3^{\ominus}$) of Sequence Polyamides containing Taurine, γ-Aminopropanesulfonic Acid (Aps) or Sulfanilic Acid in Trifluoroacetic Acid

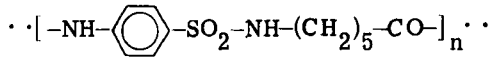
	Chemical shift δ (ppm)				
	18.24 MHz		9.12 MHz spectra		
	Aminosulfonic acid	ω-Aminocarboxylic acid	Aminosulfonic acid	ω-Aminocarboxylic acid	
$(\gamma\text{-Aps-}\beta\text{-Ala})_n$	244.73	287.63			
$(\gamma\text{-Aps-}\epsilon\text{-Aca})_n$			241.62	288.31	
$(\text{Sulf-}\epsilon\text{-Aca})_n$			239.06	308.22	
$(\text{Tau-}\epsilon\text{-Aca})_n$	249.77 ± 0.1	286.10 ± 0.1	250.27	287.10	
$(\text{Tau-}\gamma\text{-Abu})_n$	251.53	286.50			
$(\text{Tau-}\beta\text{-Ala})_n$[a]	252.63	286.88			
$(\text{Tau-Gly})_n$	260.32 ± 0.1	290.90 ± 0.1	261.02	291.90	-
$(\text{Tau-Gly-Gly})_n$			261.22	291.71	267.16
$(\text{Tau-Gly-}\beta\text{-Ala})_n$			255.16	291.88	253.07
$(\text{Tau-}\beta\text{-Ala-Gly})_n$			260.70	287.95	261.40

[a] δ = 254.46 and 273.97 ppm in H_2O; pH 13.6, at 35°C.

$$\cdots[-NH-(CH_2)_3-SO_2-NH-(CH_2)_m-CO-]_n\cdots$$

II

a: m = 2
b: m = 5



III

downfield shift of the nitrogen signals [11] (Fig. 1). This effect is more pronounced in the case of the nylons, since $(Gly)_n$ and $(\epsilon\text{-Aca})_n$ show a shift difference of ca. 30 ppm, while there is a shift difference of only ca. 5 ppm (Fig. 1 and Table 1) for the amino acyl units in Ia and Id. However, the nitrogen of the tauryl residue is also affected by the chain length of the ω-amino acyl units and shows a shift difference of ca. 10 ppm if Ia and Id are compared. This spectroscopic behavior is interesting because it is contrary to the expected direct relationship between electron density and chemical shift. Increasing chain length results in a better separation of the two electron withdrawing functional groups, so that the electron density of both the amino and the carbonyl group increases, as known for the pK values of the ω-amino acids [14]. Hence we must conclude that the nylons as well as the polyamides Ia-d and IIa, b present a

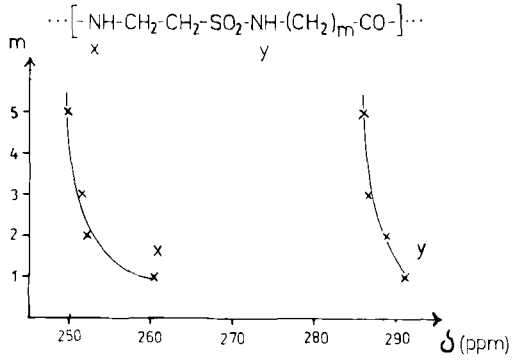


FIG. 1. ^{15}N-NMR chemical shifts (ppm relative to external NO_3) of taurine containing binary sequence polyamides.

reciprocal electron density-chemical shift relationship. Further observations give evidence that such reciprocal relationships are quite common in the ^{15}N-NMR spectroscopy of ω-amino acid derivatives and sulfonamides. Thus, the signal of the ω-aminopropanesulfonyl residue in IIa and b is ca. 7 ppm downfield from that of taurine, although the nitrogen is more removed from the strongly electronegative sulfonyl group. The most striking example is the high field shift of the sulfonamide groups themselves. The sulfonamide nitrogens are known to possess a much lower electron density than normal amides.

On the other hand, the extent of the downfield shift is certainly dependent on solvation. It is known from studies of oligopeptides and polysarcosine [9, 15] that protic and acidic solvents produce downfield shifts when compared with aprotic or less acidic solvents. Also, theoretical arguments indicate that protonation or hydrogen bond formation at an amide carbonyl group should cause a downfield shift of the ^{15}N-NMR signal [16]. Hence, an increase in the aliphatic chain length of a monomer unit results in increased basicity of the amide group, stronger protonation or hydrogen bond formation in acidic solvents, and consequently a greater downfield ^{15}N chemical shift. Moreover, a series of amide groups should shift more downfield with increasing chain length if it is more basic than a comparable series of other amide groups. Indeed, this spectroscopic behavior is found, if nylons 2 to 6 are compared with the less basic amide groups of Ia-d (x in Fig. 1) [11] and when these are in turn compared with the sulfonamide groups in Ia-d (y in Fig. 1). In this connection it is noteworthy that the carbonyl signals in the ^{13}C-NMR spectra of nylons 2 to 6 and Ia-d show an analogous downfield shift behavior. A more detailed investigation of solvation and chemical shift of amino acid derivatives and polypeptides will shed more light on this problem.

Isomeric Sequences

Polymers with isomeric sequences are useful model compounds for spectroscopic investigations for two reasons: (1) they allow us to test the sensitivity of a given method to variations in the primary structure, and (2) they allow us to study neighboring residues effects.

In a previous paper we defined sequence isomerism of first and second order [12]. The sequences $(\text{-}\gamma\text{-Aps-}\beta\text{-Ala})_n$ (IIa) and $(\text{-Tau-}\gamma\text{-Abu-})_n$ (Ic) are examples of the former case, because the sequence units are isomeric, while the monomer units possess different structures. Since the sulfonamide signals are considerably high-field shifted from those of carboxylic acid amides and since

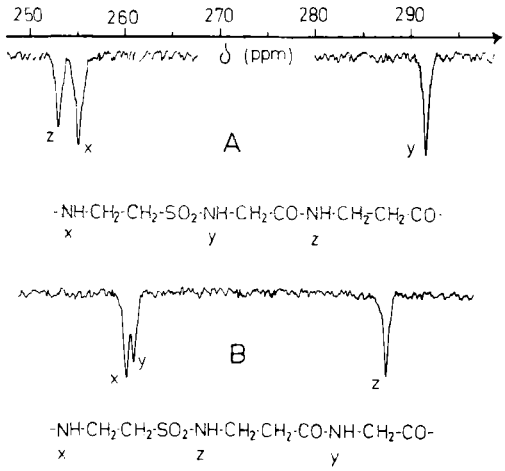


FIG. 2. 9.12 MHz ^{15}N-NMR spectra of isomeric sequence polypeptides in trifluoroacetic acid: (A) (Tau-Gly-β-Ala)$_n$ (IV); (B) (Tau-β-Ala-Gly)$_n$ (V).

the shift of ω-aminosulfonic acid derivatives is dependent on the monomer chain length, as discussed above, it is not surprising that the two isomeric sequences exhibit different spectra (Table 1).

More interesting is the spectroscopic behavior of (Tau-Gly-β-Ala)$_n$ (IV) and (Tau-β-Ala-Gly)$_n$ (V), two isomeric sequences built up of identical monomer units [12]. Figure 2 clearly shows that the different positions of the monomer units relative to each other results in different ^{15}N-NMR spectra. This observation, in addition to similar results reported in other papers of this series, demonstrates the usefulness of ^{15}N-NMR spectroscopy for the characterization of sequence polymers or for a sequence analysis of random copolypeptides and copolyamides. Whether or not a small perturbation of a regular sequence is detected depends, of course, on the signal-to-noise ratio of the spectrum. Within the S/N limitations all ^{15}N-NMR spectra presented in this paper were in full agreement with unperturbed alternating sequences.

$$[-NH-CH_2-CH_2-SO_2-NH-CH_2-CO-NH-CH_2-CH_2-CO-]_n$$

IV

$$[-NH-CH_2-CH_2-SO_2-NH-CH_2-CH_2-CO-NH-CH_2-CO-]_n$$

V

$$\overset{\oplus}{NH_3}-CH_2-CH_2-SO_2-NH-CH_2-CO-NH-CH_2-CH_2-CO-O^{\ominus}$$

VI

$$\overset{\oplus}{NH_3}-CH_2-CH_2-SO_2-NH-CH_2-CH_2-CO-NH-CH_2-CO-O^{\ominus}$$

VII

The neighboring residue effects observed in the spectra of IV and V can be interpreted by comparison of both spectra with those of binary sequence polymers. Thus, we know from $(Tau\text{-}Gly)_n$, (Ia) and $(Tau\text{-}\beta\text{-}Ala)_n$ (Ib) that the taurine nitrogen acylated by glycine is 7.5 ppm upfield from that acylated by β-alanine. A similar, but not identical shift difference ($\Delta\delta_N$ = 5.5 ppm) is observed for IV and V. Analogously to Ia and Ib, the signal of the Tau-Gly bond in IV appears 4 ppm upfield from that of the Tau-β-Ala bond in V. Furthermore, from $(\beta\text{-}Ala)_n$ and $(\beta\text{-}Ala\text{-}Gly\text{-}Gly)_n$ we know that the signal of a β-Ala-Gly bond is 6 ppm downfield from that of a Gly-Gly bond and the signal of a Gly-β-Ala bond 6 ppm highfield from that of a β-Ala-β-Ala bond. These shift differences are likewise found in the spectra of IV and V. Thus, we may conclude that the shift of a particular nitrogen in polypeptides and copolyamides is predominantly determined by the nature of the two adjoining monomer units. However, the comparison of Ia and Ib on the one hand and IV and V on the other hand indicates that nighboring residue effects of higher order (sequence effects) can exist. Although such sequence effects are usually small (0-2 ppm), they are highly interesting, since they allow the sequence analysis of copolymers with more than three monomer units.

Neighboring residue effects in ^{15}N-NMR spectra of oligopeptides were first studied by Roberts and co-workers [17] on aqueous solutions of dipeptides. Since dipeptides in water possess two charged, strongly solvated endgroups, it is at least doubtful that they are useful models for polypeptides and proteins. The neighboring residue effects are probably masked by solvation and end-group effects as postulated by Hawkes and Randall [15, 18].

In a previous paper we reported a comparison of two tripeptides with the corresponding sequence polypeptides in trifluoroacetic acid [11]. Although the tripeptides possess only one charged end-group under these conditions, it turned out that the shifts of their amide nitrogens differ by about 0.5-1.5 ppm from those of the polypeptides. An analogous comparison was made in this work by using the tripeptides VI and VII for which the following chemical shifts (relative

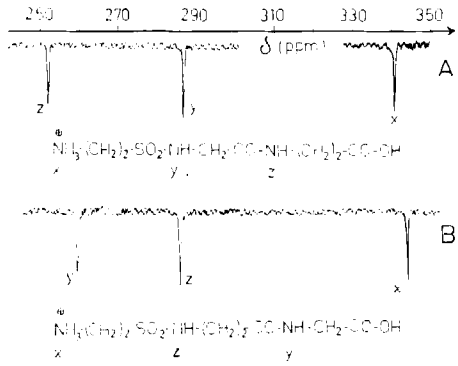


FIG. 3. 9.12 MHz [15] N-NMR spectra of tripeptides in trifluoroacetic acid: (A) H-Tau-Gly-β-Ala-OH; (B) H-Tau-β-Ala-Gly-OH.

to external $NO_3^{\ominus}$) were found in their 9.12 MHz spectra (Fig. 3): VI, δ = 256.71 ppm (Gly-β-Ala); 291.68 ppm (Tau-Gly); 345.86 ppm ($\overset{\oplus}{N}H_3$); VII, δ = 261.37 ppm (β-Ala-Gly); 287.87 ppm (Tau-β-Ala); 346.23 ppm ($\overset{\oplus}{N}H_3$). The comparison with the spectra of IV and V (Table 1) shows nearly identical shifts (± 0.1 ppm) for three amide groups; however, the Gly-β-Ala signal of VI appears ca. 3 ppm upfield from that of IV. Thus we come to the same conclusion as in our previous paper: the neighboring residue effects are qualitatively identical for both tripeptides and sequence polymers. However, the unprotected oligomers are not reliable models for polypeptides. At least tetrapeptides or fully protected tripeptides seem to be required as useful models for polypeptides and proteins.

N–H Deprotonation

Alkyl sulfonamides are known to be soluble in alkaline water, since the N–H acidity is sufficiently high to allow deprotonation. The alternating polysulfonamides Ia-d, IIa, b, and III show similar behavior; thus, they become soluble in water at pH > 12. Since the deprotonation results in a negative charge at the nitrogen, a strong highfield shift was expected for the sulfonamide signal in alkaline solution. However, the comparison of $(Tau\text{-}\beta\text{-}Ala)_n$ in trifluoroacetic acid and in water at pH 13.6 (Fig. 4) clearly demonstrates that deprotonation leads to a substantial downfield shift. Thus, we find

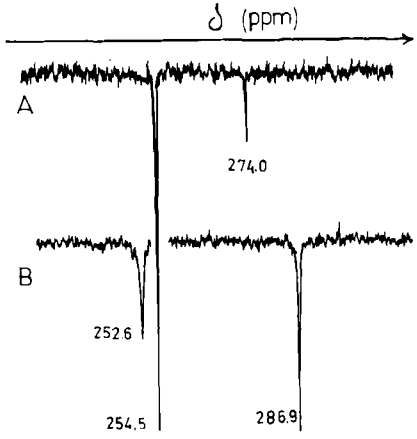


FIG. 4. 18.24 MHz ^{15}N-NMR spectra of (Tau-β-Ala)$_n$ in: (A) water at pH 13.6; (B) trifluoroacetic acid.

again a reciprocal relationship between electron density and chemical shift. It is not probable that this downfield shift results from a general solvent effect, since it is known from several oligo- and polypeptides that less acidic solvents cause small upfield shifts compared with the more acidic ones [9, 15, 21]. In agreement with this general rule, the amide signal of Ib appears in water ca. 2 ppm upfield from the corresponding signal in trifluoroacetic acid (Fig. 4).

Another interesting effect concerns the intensity of the sulfonamide signal which is substantially reduced in alkaline water. Two reasons may be advanced to explain this finding: (1) a reduced nuclear Overhauser effect resulting from the influence of paramagnetic cations on the relaxation of the nitrogen anion; (2) saturation of the signal resulting from an increased relaxation time due to the lack of protons. It is known from investigations on the ^{15}N-NMR spectra of glycine [19, 20] poly-L-lysine and isopoly-L-lysine [21] in water at various pH's that traces of paramagnetic ions may influence strongly the nuclear Overhauser effect of ^{15}N nuclei. The low concentration of paramagnetic ions always present in normal water or in walls of new glassware may be sufficient for such an influence. In alkaline water the sulfonamide nitrogen must be several orders of magnitude more sensitive to the influence of cations, because the nitrogen anion is a much better ligand than a protonated, neutral nitrogen. Although we feel that the observed decrease in signal intensity is chiefly caused by paramagnetic ions, we cannot rule out that saturation of the signal is likewise involved. Further investigations on the spectroscopic

behavior of sulfonamides in various solvents are required to clarify the situation.

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