Polymer Bulletin 6, 101–108 (1981)

Polymer Bulletin

Secondary Structures of Peptides

2. Detection and Quantification of Secondary Structures of Solid Polypeptides by Means of ^{13}C-NMR CP/MAS Spectroscopy

Detlef Müller[1] and Hans R. Kricheldorf[2]

[1] Bruker Analytische Meßtechnik GmbH, D-7512 Rheinstetten, Federal Republic of Germany
[2] Institut für Makromolekulare Chemie der Universität, Stefan-Meier-Straße 31, D-7800 Freiburg, Federal Republic of Germany

Herrn Prof. Dr. H. Zahn zum 65. Geburtstag gewidmet

SUMMARY

75.46 MHz ^{13}C-NMR CP/MAS spectra of solid poly(l-alanines) with various degrees of polymerization were measured. The signals of all three carbons are split into two peaks. One peak presents the α-helix structure and one the pleated sheet form. Equimolar mixtures of helical poly(L-alanine) with poly(glycine) and poly(L-valine) were measured under various conditions. It was found that the peak areas are proportional to the mole ratios of the different secondary structures and neither a mismatch of the Hartmann-Hahn conditions nor variations of the mixing time or the pulse interval (relaxation delay) have a significant influence on the intensity ratios. Hence, a quantitative evaluation of the ^{13}C NMR spectra with respect to the secondary structure is feasible.

INTRODUCTION

The secondary structure of synthetic homo- and copolypeptides is of great interest to polymer chemists and biochemists, because polypeptides are useful models of proteins and because their usefulness as synthetic fibers depends on the secondary structure of both starting material and product[1)]. X-ray diffraction and IR spectroscopy have been used so far to elucidate the conformation of solid polypeptides; yet, both mothods suffer from severe short-comings. IR-bands, such as the Amide-I band, are in many cases broad and not well resolved (e.g. in the case of poly(D,L-amino acids)). They dont allow a quantification of the molar fractions when a sample consists of two different secondary structures. The X-ray diffraction pattern of most polypeptides only show one or two signals, α- and ß-structures may give similar signal pattern[2)] and a quantitative evaluation of the mostly diffuse signals is not reliable. Hence, a third method which allows an unambiguous identification of different secondary structures along with their

0170-0839/81/0006/0101/$01.60

quantification is highly desirable. In the first part of the present series we have investigated whether ^{13}C-NMR CP/MAS spectra allow a qualitative and quantitative analysis of the secondary structure of polyalanines.

RESULTS and DISCUSSION

Using IR spectra and X-ray diffraction Kawai, Komoto et al. have shown that poly(L-alanine) obtained by polymerization of L-alanine-N-carboxyanhydride (L-Ala-NCA) possess both types of secondary structures α-helices and pleated sheets[3,4]. With increasing degree of polymerization (DP) the helical fraction increases at the expense of the non helical fraction; yet, a quantitative determination of the mole fraction was not feasible. Using benzylamine as initiator we have also synthesized poly(L-alanines) of various DPs. It is well known that primary amine-initiated polymerizations of NCAs have living character, so that the DP is determined by the monomer/initiator ratio and by the conversion. The samples used for the measurements of Fig. 1 A-C were obtained in this way with M/I ratios = 10, 20 or 50 and conversions of 98-100%. Figs. 1 A-C demonstrate that all three signals are split into two peaks representing the α-helix and the pleated sheet (ß) structure. With increasing DP the intensity of one set of peaks decreases strongly, and thus, was attributed to the pleated sheet (ß) structure. This assignment was confirmed by FT-IR spectra which provided a clear separation of two Amide-I bands. The α-helix bond at 1660 cm^{-1} and the pleated sheet band at 1633 cm^{-1} have nearly equal intensities, when the sample with DP=10 is measured (Fig. 2 A) while the samples with DP = 20 and 50 (Fig. 2 B) show a stronger α-helix band.

The signal intensities of the expanded spectra of Figs. 1 A-C were quantified by means of a curve resolver. In this way 48 ± 5 % α-helix was found at $\overline{DP}$ = 10, 75 ± 3 % α-helix at $\overline{DP}$ = 20 and 90 ± 2 % α-helix at $\overline{DP}$ = 50. When L-Ala-NCA was polymerized with triethylamine in dioxane at 20°C a sample with $\overline{DP} > 100$ was obtained. The ^{13}C NMR CP/MAS spectrum allowed us to detect in this case still a fraction of 3 ± 1 % ß-structure.

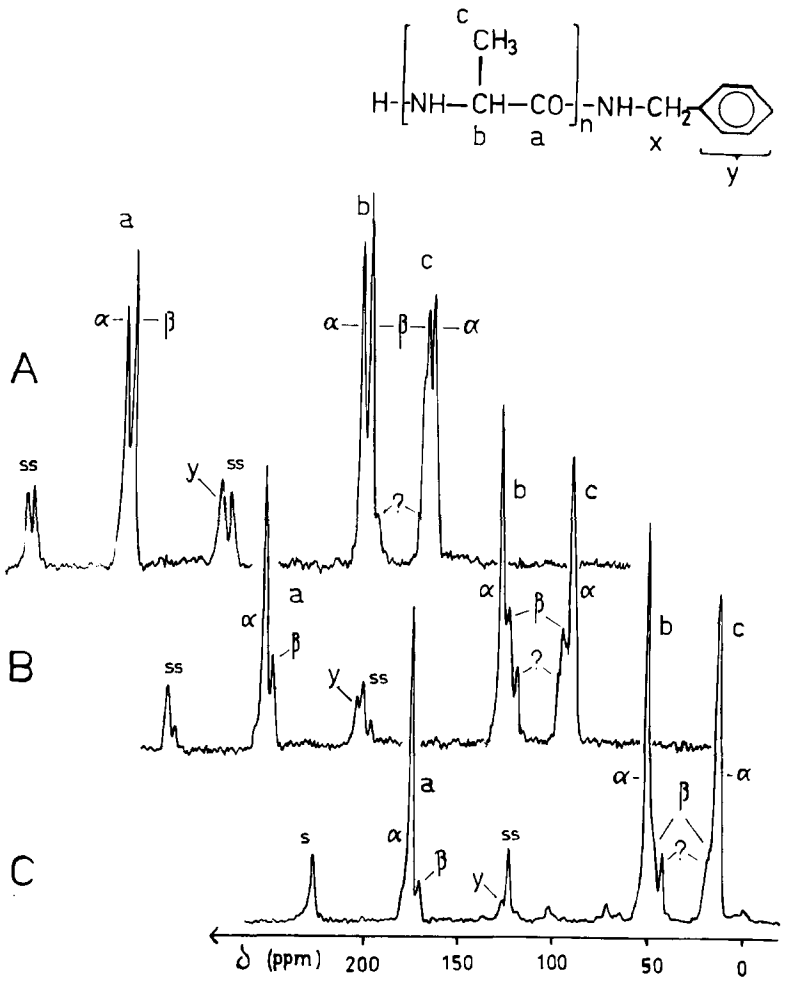

Fig.1 75.46 MHz ^{13}C NMR CP/MAS spectra of poly(L-alanines) obtained by benzylamine-initiated polymerizations in acetonitrile at 20°C: A) $\overline{DP}$ = 10; B) $\overline{DP}$ = 20; C) $\overline{DP}$ = 50.

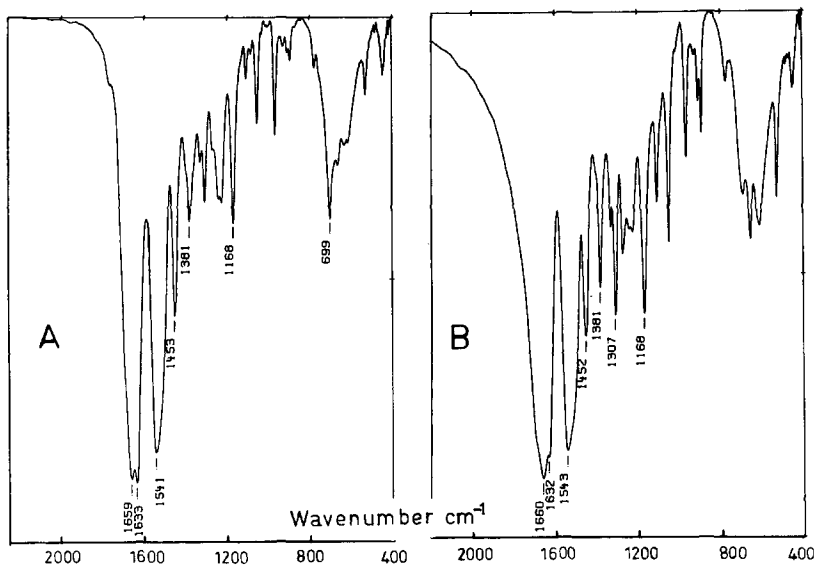

Fig.2 FT-IR spectra of poly(L-alanines) in KBr: A) DP = 10; B) DP = 50; same samples as in Fig. 1.

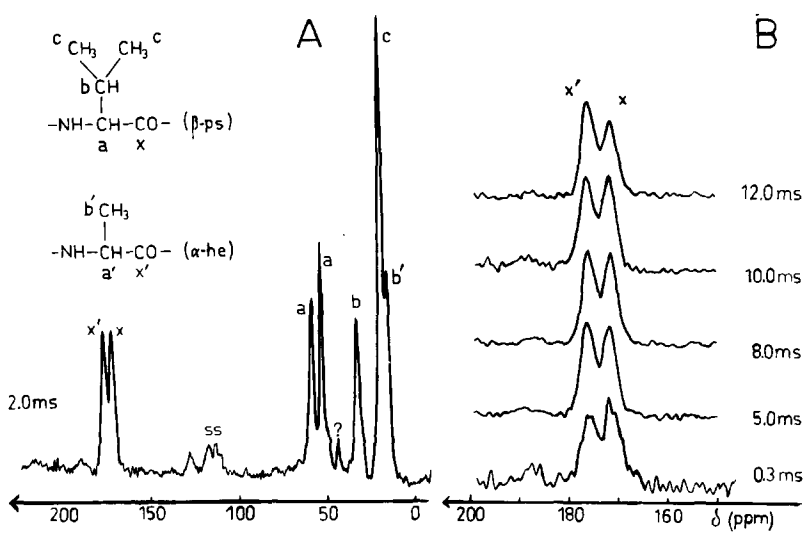

Fig.3 75.46 MHz ^{13}C NMR CP/MAS spectra of an $(L\text{-}Ala)_n/(L\text{-}Val)_{50}$ mixture (mole ratio 1:1): A) complete spectrum, contact time 1 ms; B) carbonyl signals only, various contact times.

In order to test whether the intensities of the α- and ß-peaks are really proportional to the mole fractions of the corresponding secondary structures two series of measurements were conducted. The $(L\text{-}Ala)_n$ sample with 97 ± 1 % α-helix content was mixed with equimolar amounts of $(L\text{-}Val)_{50}$ or $(Gly)_{50}$, because both polypeptides dont contain any α-helices but only ß-structures. When measured under normal conditions (contact time: 1-3 ms; pulse interval: 4-8 s) the intensity ratios of the two carbonyl signals equaled unity (± 0.05). Also the α-carbons of the $(L\text{-}Ala)_n/(L\text{-}Val)_{50}$ mixture gave signals of equal intensities. This result is important because it demonstrates that at least the peak intensities of CO-signals really parallel the mole fractions of α- and ß-structures regardless of the nature of the peptides under investigation. Also the signal intensities of α-carbons may be used for a quantification of secondary structures if the number of substituents is identical. This means, that in principle also quantitative investigations of the secondary structures of copolypeptides and proteins are feasible.

Furthermore we have studied the influence of acquisition parameters on the intensity ratios of α- and ß-peaks.The $(L\text{-}Ala)_n/(L\text{-}Val)_{50}$ mixture was measured with various contact times (Fig. 3 A, B). Only at very short ($\leq$ 0.3 ms) or very long ($\geq$ 12 ms) contact times the intensity ratios deviate from unity. At short contact times the $(L\text{-}Val)_{50}$ CO-signal is more intensive, at long contact times the $(L\text{-}Ala)_n$ CO-signal. This relationship is presumably a consequence of the fact that the Val residue contains twice as many protons, as the Ala unit, so that the polarization transfer to the carbonyl-C is more effective in the case of $(L\text{-}Val)_{50}$. Another potential explanation is a shorter crosspolarization time T_{CH}, due to a different local environment of the protons in $(Val)_n$ and $(Ala)_n$. As a consequence the Val-CO signal grows faster at short contact times while it decreases faster at long contact times following the decreasing polarization (due to rotating frame relaxation) of the protons. Furthermore, we have produced a mismatch of the Hartmann-Hahn conditions with $\gamma_H \cdot H_1$ ca. 30 %

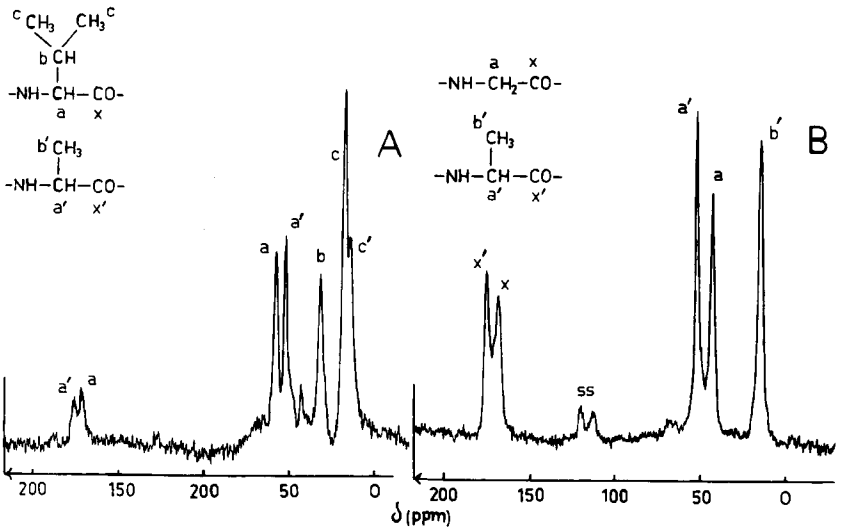

Fig.4 75.46 MHz ^{13}C NMR CP/MAS spectra of A) an $(L\text{-Ala})_n/(L\text{-Val})_{50}$ mixture (1:1); mismatch of the Hartmann-Hahn conditions; B) an $(L\text{-Ala})_n/(Gly)_{50}$ mixture (1:1), rep.time 4 s.

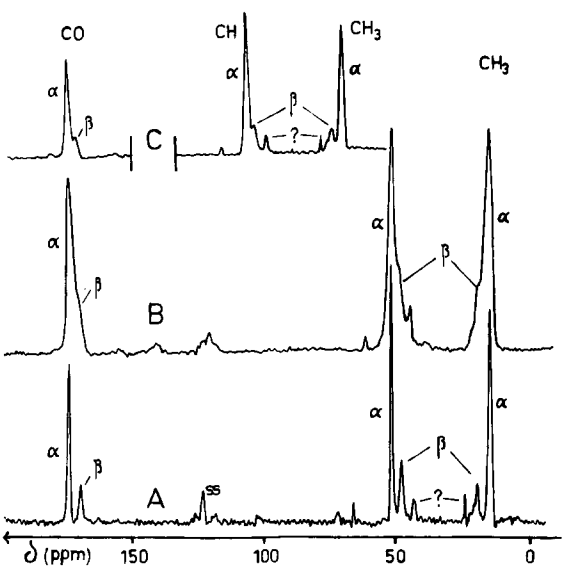

Fig.5 ^{13}C NMR CP/MAS spectra of $(L\text{-Ala})_{30}$: A) 75.46 MHz, resolution enhanced ; B) 50.35 MHz, normal resolution; C) 50.35 MHz, resolution enhanced.

below its optimum value. The main consequence of this measure are strongly reduced intensities of the carbonyl signals. However, their intensity ratio deviates only slightly from unity and that of the α-carbons equals exactly unity (Fig. 4 A). In the case of the $(L\text{-Ala})_n/(Gly)_{50}$ mixture we have varied the repetition time (and thus, the relaxation delay of the protons) from 1 to 30 s. Again, the intensity ratios of α- and ß- peaks were not affected, the only consequence being an overall decrease of the signal-to-noise ratio at short repetition times (<3 s).

The above measurements demonstrate that the quantification of ^{13}C-NMR spectra of solid polypeptides depends mainly on the signal-to-noise ratio and on the resolution of α- and ß-peaks. Hence, we have also looked at the problem of insufficient resolution. Fig. 5 A demonstrates that in the case of $(L\text{-Ala})_{20}$ a 50.35 MHz spectrum suffices to detect the presence of pleated sheets from shoulders of all three signals (Fig. 5 A). A quantitative evaluation is not feasible unless strong resolution enhancement is applied which makes individual ß-peaks detectable. (Fig. 6 B). Yet, this measure requires an excellent signal-to-noise ratio, e.g. 10 000 transients in Figs. 5 A, B. Similar resolution enhancement applied to the 75.46 MHz spectrum not only requires less transients (e.g. 1000 in Fig. 5 C) but also yields separate α- and ß-peaks for all three signals (Fig. 5 C). On the basis of these results it is obvious that 22.6 and 25.0 MHz ^{13}C NMR spectra are in most cases not appropriate for investigations of the secondary structure of solid polypeptides.

Finally, it is to be noted that all spectra of poly(alanines) as well as those of other polypeptides with ß-structures display weak, but sharp signals at 44.2 and 22.3 ppm. The origin of these signals is not yet clear. A more detailed discussion will be presented in another part of this series.

MEASUREMENTS

The 50.33 MHz spectrum of Fig. 5 A, B was obtained on a Bruker CXP-200, all other spectra on a Bruker CXP-300 FT-Spectrometer. Samples of 150-200 mg were measured in 6.3 mm i.d. rotors made of deuterated PMMA at a spinning rate of ca. 4 KHz. CP/MAS was used with alternation of the proton 90^{o} pulse phase. Proton 90^{o} pulse length was between 3 and 4.5 μs, corresponding to an H_1 field strength of 57 and 85 KHz which was found to be adequate for this class of compounds. Normally, the contact times were 3 ms and the repetition times 4-6 s. Mathematical sideband removal was not applied, in order to maintain the true lineshape. The variation of the magic angle was checked with glycine between the measurements; the linewidth of the carbonyl signal never exceeded 30 Hz. The chemical shifts were referenced to TMS by using the carbonyl signal of glycine as secondary standard (170.09 ppm). The inherent stability of cryomagnets allows measurements without lock or internal reference standard.

REFERENCES

1) J.Noguchi, S.Tokura and N.Nishi; Angew. Makromol.Chem. 22 107 (1972)
2) T.Komoto, K.Y.Kim, M.Oya and T.Kawai; Makromol. Chem. 169 301 (1973)
3) A.Fuije, T.Komoto, M.Oya and T.Kawai; Makromol. Chem. 169 301 (1973)
4) T.Komoto and T.Kawai; Makromol. Chem. 172 221 (1973)

Received November 9, accepted November 12, 1981