Polymer Bulletin 8, 495–502 (1982)

Polymer Bulletin

Secondary Structure of Peptides

7. Induction of Secondary Structure in Solid Block Copolypeptides Studied by Means of ^{13}C NMR CP/MAS Spectroscopy

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SUMMARY

Various copolypeptides with a two block sequence are prepared by batchwise copolymerizations of amino acid N-carboxyanhydrides (NCAs) using primary amines as initiators. An α-helix forming amino acid and a ß-sheet forming amino acid were combined in the two blocks and the mutual influence of the two secondary structures was investigated by means of ^{13}C NMR cross-polarization magic-angle spinning (CP/MAS) spectra. It was found that the second (succeeding) block adopts partially the secondary structure of the first block.

INTRODUCTION

In previous papers of this series we have demonstrated that ^{13}C NMR CP/MAS spectra allow the identification of the secondary structure of almost all common homopolypeptides[1-4]. By applying suitable acquisition parameters it is also feasible to quantify the signal intensities and to determine the mole fractions, when two different secondary structures are simultaneously present. Furthermore, the chemical shifts of the homopolypeptides suggest [2] that it will be possible in favourable cases to determine the secondary structure of individual amino acids in binary copolypeptides. The present study was undertaken to investigate whether ^{13}C NMR CP/MAS spectra allow one to analyze the secondary structure of binary block copolypeptides.

RESULTS and DISCUSSION

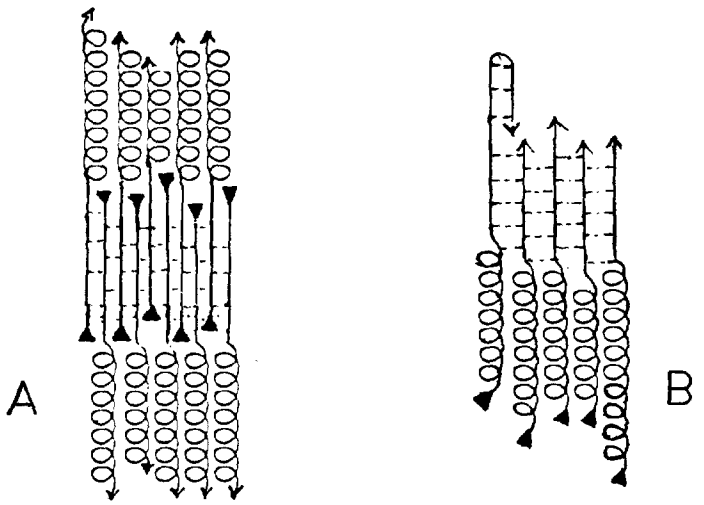
Primary amin-initiated polymerizations of α-amino acid NCAs behave, for monomer/initiator ratios $\leq$ 100 like living

0170-0839/82/0008/0495/$01.60

Table I. Reaction conditions and results of benzylamine-initiated block-copolymerizations of various α-amino acid NCAs at 25°C

No.	Mon. 1 NCA of	Mon. 2 NCA of	Mon./Init. Mon.1/Mon.2	Solvent	Time (d)	Yield (%)	% α-helix Mon. 1	% α-helix Mon. 2
1	Gly	L-Ala	20:1/40:1	DMF [a]	1/4	79 [b]	0	70-75
2	Gly	L-Leu	20:1/20:1	DMF [a]	1/4	80 [c]	0	60-65
3	L-Val	L-Ala	20:1/20:1	Dioxane	2/4	100	ca.10?	55-60
4	L-Val	Gly	50:1/25:1	Acetonitrile	2/2	86 [d]	80-85	ca.20?
5	L-Ala	L-Val	50:1/50:1	DMF [a]	2/4	64 [e]	80-85	25-30
6	L-Ala	L-Val	50:1/50:1	Dioxane	2/4	100	85-90	10-15
7	L-Leu	Gly	50:1/50:1	Acetonitrile	2/4	100	80-90	ca.20?
8	L-Leu	L-Val	50:1/50:1	DMF [a]	2/4	60 [f]	60-70	45-50
9	γOBzl-L-Glu	L-Ala	10:1/10:1	CH_2Cl_2	1/2	98	80-90	85-90

a) Dimethylformamide
b) ca. 70 % of Ala-NCA
c) ca. 70 % of Leu-NCA
d) ca. 70 % of Gly-NCA
e) ca. 40 % of Val-NCA
f) ca. 20 % of Val-NCA



Scheme I. Secondary structure of block copolypeptides built up of α-helix and ß-sheet forming amino acids (▲ = initiator)

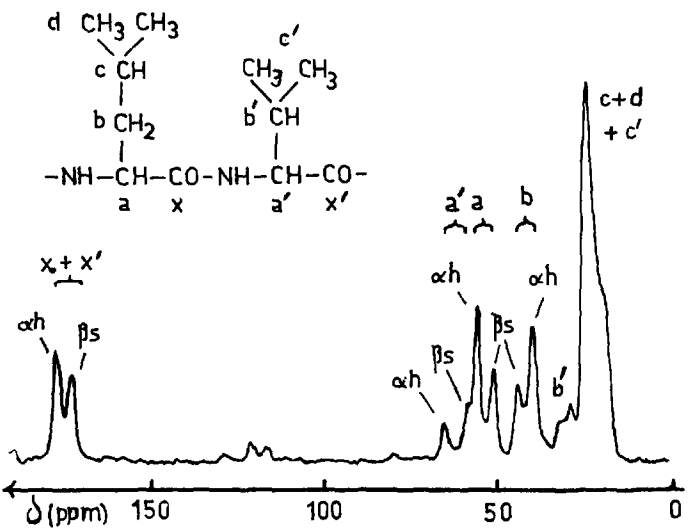


Fig. 1 75.4 MHz ^{13}C NMR CP/MAS spectrum of Bzl(Leu)$_{50}$(Val)$_{20}$ as polymerized (No. 8, Table I)

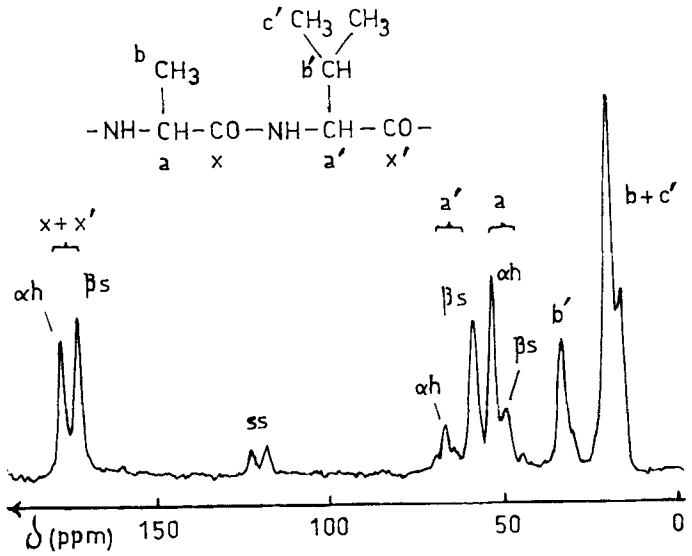


Fig. 2. 75.4 MHz ^{13}C NMR CP/MAS spectrum of Bzl(Ala)$_{20}$(Val)$_{20}$ as polymerized (No. 6, Table I)

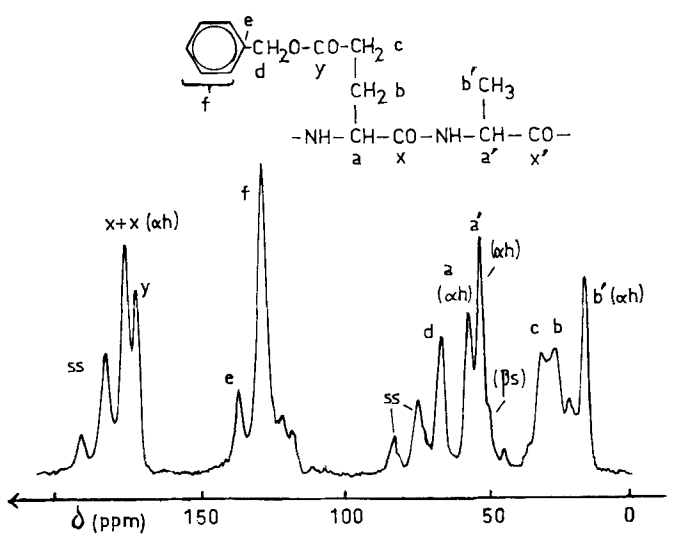


Fig. 3. 75.4 MHz ^{13}C NMR CP/MAS spectrum of Bzl(γOBzl-Glu)$_{10}$-(Ala)$_{10}$ as polymerized (No. 9, Table I)

anionic polymerizations of vinyl monomers. When aliphatic amines are used, the initiation is faster than the propagation. Thus, after complete conversion of the monomer the average degree of polymerization ($\overline{DP}$) equals the monomer/initiator ratio. Since the amino endgroups are still reactive (living), addition of a second NCA allows the synthesis of two-block copolypeptides. When the two amino acids used for such a batchwise copolymerization tend to adopt different secondary structures, the blocky character of the primary structure might result in a blocky structure of the secondary structure. As demonstrated in scheme I A and B, the synthesis of α-helix/ß-sheet block structures can be attempted in two ways, depending on whether an α-helix or the ß-sheet forming NCA is first polymerized. In this regard it is to be emphasized that scheme I is a simplification because the antiparallel ß-sheet of I A may contain a fraction of parallel or folded antiparallel ß-sheets while the ß-sheets of I B might contain large fractions of folded antiparallel chain instead of the schematically shown parallel structure. However, because the ^{13}C NMR CP/MAS spectra do not allow a differentiation between these different types of ß-sheets a detailed discussion of this aspect is not necessary.

The reaction conditions and results obtained by batchwise copolymerizations of various NCAs are summarized in Table I. Gly-NCA and Val-NCA were used as ß-sheet forming monomers, while L-Ala-NCA, L-Leu-NCA and γOBzl-L-Glu-NCA were the helicogenic monomers. When Gly-NCA and L-Val-NCA were used as first monomers (Nos. 1-3, Table I),we find that the alanine and leucine blocks formed by subsequent copolymerization contain 30-40 % ß-sheet structure. A similar percentage of ß-sheet structure is also found when primary amine-initiated polymerizations of L-Ala- or L-Leu-NCA are conducted with monomer/initiator ratios similar to those used in Table I (Nos. 1-3; $M/I \leqslant 40$). We have demonstrated that the ß-sheet fractions of homopolypeptides are the result of a bimodal molecular weight distribution. Only the oligomers (DPs <12) which precipitate in the initial stage of the polymerization form ß-sheets because they are too short to form α-helices. However, the copolymerizations of

Table I take a quite different course. The helicogenic monomers begin their chain growth on the surface of precipitated ß-sheet crystallites, and thus, we may conclude that this heterogeneous initiator influences the secondary structure of the growing chains. The DP of the second block at which the ß-sheet→α-helix transition occurs will depend upon the local steric conditions.

The influence of block 1 on the secondary structure of block 2 is still more obvious when block 1 is prepared from helicogenic NCAs (Nos. 4-9, Table I). For instance, when poly(L-alanine) or poly(L-leucine) is used as initiator for the polymerization of L-Val-NCA (Nos. 5, 6, 8) the resulting valine blocks are partially helical (Figures 1 and 2). The α-helix/ß-sheet ratio of the valine blocks depends on the conversion of L-Valine NCA. The low conversions observed in dimethylformamide (Nos. 5 and 8) result in a higher helix percentage of the valine blocks, because only those segments directly attached to the helical initiator are forced into the unfavourable α-helix conformation. The DP at which the αh ⟶ ßs transition occurs seems to be $\lesssim 10$ (i.e. between one and two helical loops). When Gly-NCA is used as second monomer (Nos. 4 and 7) it is difficult to determine the α-helix fraction for the following reasons. The α-C signal is not sensitive to a change of the secondary structure [2] and the α-helix peak of the CO-signal (171.5 ± 0.5 ppm) overlaps with the ß-sheet peak of the alanine or leucine CO-signals [2]. Since the leucine and alanine blocks contain a small ß-sheet fraction due to their bimodal molecular weight distribution [3,4], the problem of overlapping CO-peaks is difficult to solve. However, the intensity ratio of the ßs and αh-peaks of the CO-signals of Ala and Leu is higher than in the case of their α-C signals which do not overlap with Gly-signals. This difference indicates that the ßs peak of the Ala and Leu CO-signals is overlapping with an αh peak of the Gly blocks; yet this conclusion is speculative.

Furthermore, we have used short blocks ($\overline{DP} = 10$) of poly(γ-O-benzyl-L-glutamate) as initiator for L-Ala-NCA. Gly-NCA

and Val-NCA are useless as second monomer because their ^{13}C-NMR signals overlap with those of the (γ-OBzl-L-Glu) blocks[2]. In the case of L-alanine a high degree of helicity was determined from the α-C signal (Fig. 3) despite the low DP of 10. When primary amines are used as initiators under similar conditions, an α-helix content of only 45 - 50 % is found (s. Fig. 1 A in ref. 1). Since poly(γ-OBzl-L-Glu) undergoes a ßs $\rightarrow \alpha$h transition at DP = 7 or 8 [5-7] it can act as a helix inducing initiator even at DP = 10, and thus, considerably increase the helix content of the alanine blocks. A similar result was obtained with γ-O-methyl Glu-NCA as second monomer as described in the preceeding paper [4].

Finally, we wi h to emphasize that the present work demonstrates for the first time that ^{13}C NMR CP/MAS spectroscopy is the only method which allows one to determine the secondary structure of the two components of a binary copolypeptide separately.

EXPERIMENTAL

Polymerizations: The NCAs were prepared as described previously [2,3]. The solvents were dried over sodium or P_4O_{10} according to their chemical stability. The copolymerizations were conducted beginning with the homopolymerization of monomer 1. 30 mmol of monomer 1 was dissolved in 50 ml solvent and the initiator was added in form of a 1 M solution in dry dioxane. When the conversion of monomer 1 was complete (after 1 or 2 days) a solution of 30 mmol monomer 2 in 30 ml solvent was added and the reaction mixture was vigorously shaken in order to obtain a homogeneous suspension. When the evolution of CO_2 had completely ceased (after 2-4 days) the reaction mixture was diluted with 300 ml cold diethylether, the precipitated copolypeptide was isolated by filtration and dried at 60°C/12 mm. The completeness of the conversion of monomer 1 was checked in separate experiments.

NMR measurements: 75.4 MHz ^{13}C NMR CP/MAS spectra were obtained on a Bruker CXP-300 T-spectrometer at spinning rates of 3.9 -4.1 KHz in rotors made from deuterated poly(methylmethacrylate). CP/MAS was conducted with a single contact pulse sequence and alternation of the ^{1}H 90° pulse phase. The proton 90° pulse lengths was 3 μs corresponding to a $\gamma^{1}H$ field strengths of 57 KHz. A contact time of 1 ms and a repetition time of 4 s was used in all cases. The magic angle was checked by means of glycine between the measurements. 300 - 500 trasients were accumulated for the individual measurements. The assignments of the signals are based on comparison with the spectra of homopolypeptides[2].

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Received September 16, accepted October 4, 1982