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Secondary Structure of Peptides

6. ^{13}C NMR CP/MAS Investigation of Solid Poly(γO-Methyl-L-Glutamate) and Poly(γO-Benzyl-L-Glutamate) Prepared from N-Carboxyanhydrides (NCAs)

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SUMMARY

75.4 MHz ^{13}C NMR CP/MAS spectra allowed the quantification of the secondary structure of $(\gamma OMe\text{-}L\text{-}Glu)_n$ and $(\gamma OBzl\text{-}L\text{-}Glu)_n$. $(\gamma OMe\text{-}L\text{-}Glu)_n$ contains α-helix and ß-sheet structures the ratio of which varies considerably with the average degree of polymerization ($\overline{DP}$) of the samples and with the nature of the reaction medium. At $\overline{DP}$'s ≧ 20 $(\gamma OBzl\text{-}L\text{-}Glu)_n$ contains more than 95% α-helix structure regardless of $\overline{DP}$ and reaction medium. The difference between both polyglutamates is explained by different molecular weight distributions resulting from aggregation and precipitation of oligo $(\gamma OMe\text{-}L\text{-}Glu)_n$ in the ß-sheet form.

INTRODUCTION

Secondary structure and other properties of $(\gamma OMe\text{-}L\text{-}Glu)_n$ in solution have been the object of numerous investigations. However, to the best of our knowledge the influence of the reaction conditions of NCA poly(glutamates) has never been investigated. Because we could demonstrate that ^{13}C NMR CP/MAS allow both a qualitative and a quantitative analysis of the secondary structure of almost all polypeptides [1-4] this method was preferentially used in the present work.

RESULTS and DISCUSSION

The signal assignments of the ^{13}C NMR/MAS spectra of both $(\gamma OMe\text{-}L\text{-}Glu)_n$ and $(\gamma OBzl\text{-}L\text{-}Glu)_n$ were reported previously [2]. Only peptide-CO, α-C and ß-C signals are sensitive to the nature of the secondary structure. Because the side chain CO-signal (y) overlaps with the ß-sheet peak of the peptide CO-signal (x) and the CH_3 signal (d) with the ß-sheet peak of the

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α-C signal (a, Fig. 1 A-C), the α-helix content of (γOMe-L-Glu)$_n$ must be calculated from equation 1:

$$\% \ \alpha\text{-helix} = \frac{I_{\alpha h} \cdot 100}{\frac{1}{2}\ (I_{\alpha h} + I_t)} \qquad (1)$$

I_h = intensity of an α-helix main chain signal
I_t = total intensity of an overlapping side chain signal + a ß-sheet main chain signal

In the case of (γOBzl-L-Glu)$_n$ the α-helix content is easily and more accurately determinable from the αh and ßs peaks of the α-C-signal which do not overlap with other signals (Fig.2).

In order to obtain correct intensity ratios repetition time and contact time were optimized. As optimum repetition time a value of 4s was found in agreement with other polypeptides [1]. Optimum contact times are 0.6 - 0.8 ms for (γOMe-L-Glu)$_n$ and 0.8 - 1.0 ms for (γOBzl-L-Glu)$_n$. These relatively short contact times were chosen for two reasons. First, the spin-lock proton relaxation times ($T_{1\varrho}$) of both poly(glutamates) are shorter than those of other polypeptides (4-16 ms). Second, the $T_{1\varrho}$'s of side chain and main chain protons of (γOMe-L-Glu)$_n$ differ largely. A more detailed discussion of these measurements will be published separately.

$$\overline{DP} = \frac{\text{NCA} \cdot \% \text{ conversion}}{\text{In} \cdot 100} \qquad (2)$$

Most polymerizations of γOMe-L-Glu-NCA and γOBzl-L-Glu-NCA were initiated by primary amines, because such polymerizations have a living character for $\frac{M}{I}$ ratios $\leqq$100. So that the $\overline{DP}$ can be varied and calculated according to equation (2). Samples Nos. 1-3, Table I, demonstrate that the αh/ßs ratio increases with increasing $\overline{DP}$ in analogy with poly(L-alanine) [1] and poly(L-leucine) [4] (Fig. 1 A, B). Nevertheless, the α-helix percentage of the (γOMe-L-Glu)$_n$ samples are substantially lower than those of (L-Ala)$_n$ or (L-Leu)$_n$ prepared under identical

conditions. Interestingly, they are very similar to those of poly(D-norvaline)$_n$ [4], a polypeptide which also possesses an unbranched aliphatic side chain. When the solvent was varied, the α-helix content also varied considerably (Nos. 2,4,5,6), whereas the DP remained unchanged. Such a sensitivity of the α-helix/ß-sheet ratio was never found in the case of (L-Ala)$_n$. The solvent effects on the secondary structure of (γ OMe-L-Glu)$_n$ results mainly from a better or poorer solvation of the relatively long polar side chain which influences the solubility of the oligomers. Oligomers with DP's $\lesssim 13 \pm 1$ [5,6] cannot adopt the α-helix conformation and precipitate from aprotic solvents forming antiparallel ß-sheets. Due to steric hindrance the chain growth of the ß-sheets is significantly slower than that of helical chains, so that at least a bimodal molecular weight distribution must result consistinf of oligomers of DP $\lesssim 13 \pm 1$ and helical chains with DP $\gtrsim 13 \pm 1$.

This hypothesis is supported by the following observations. For (L-Ala)$_n$ and (L-Leu)$_n$ a similar behavior was found, and experimental evidence for the bimodal MWD was obtained by extraction of the oligomers [3,4]. It is also known [7,8] that the chain growth of polypeptides which cannot form α-helices (e.g. Val, Ile, Ser, Cys) is much slower and yields lower DPs than polymerizations of α-helix forming NCAs. However, in contrast to (L-Ala)$_n$ and (γ OBzl-L-Glu)$_n$ the α-h/ßs ratio of (γ OMe-L-Glu)$_n$ is not in all cases thermodynamically controlled. After reprecipitation from trifluoroacetic acid/diethylether the α-helix content of samples Nos. 1-6 (Tab.I) was 20-30 % higher, but remained constant upon repeated reprecipitation. Obviously, a part of the original ß-sheets contains chains that are long enough to form α-helices. Hence, their formation was kinetically controlled, whereas the shorter oligomers (DPs $\lesssim 13 \pm 1$) are responsible for the thermodynamically stable ß-sheets. The latter finding contrasts sharply with the crystal growth hypothesis of Komoto and Kawai [9,10]. A more detailed investigation and a broader discussion of kinetically controlled secondary structures will be presented in Parts 7 and 11 of this series.

As a consequence of the results and hypotheses discussed above,it is expected that any measure that prevents the early precipitation of oligo (γOMe-L-Glu) must yield samples of nearly 100 % helicity. Such a measure is the use of monoamino polyethyleneoxide as initiator, because PEO is known to solubilize all kinds of oligopeptides [3,11]. The result of our experiment (No.11,Table I) confirms this expectation. Nearly 100 % helicity was also obtained with tertiary amines (Nos. 8-10) (Fig.1C). Tertiary amines initiate only a few chains simultaneously and the propagation is faster than the initiation in contrast to (aliphatic) primary amine-initiated polymerizations [7]. Hence, the actual concentration of oligomers is extremely low and the formation of high molecular weight polypeptides is favoured [12].

Finally, we have polymerized γOBzl-L-Glu-NCA in a variety of solvents (Table II). All samples having DPs $\geq$ 20 contain 90-100 % α-helix structure, and the ß-sheet peak of the α-C-signals is clearly detectable only for DP's 10 (Fig.2). Thus, the two Glu-NCAs behave quite differently and again the solubility of the oligomers (and polymers) is the key to a correct interpretation. Whereas all polymerizations of γOMe-L-Glu-NCA showed a heterogeneous course, we never could observe precipitation of oligomeric (γOBzl-L-Glu)$_n$ from the reaction mixtures. This observation agrees well with the good solubility of (γOBzl-L-Glu)$_n$ in most organic solvents which is the main reason for the numerous physicochemical investigations of this polypeptide. When a Poisson molecular weight distribution is considered, as is expected for a living polymer in homogeneous solution, (the experimental data found in dioxane slightly deviate from this simplified consideration [13]), the ca.20 % α-helix content found at $\overline{DP}$ = 10 (Nos. 1,4, Table II) suggests that the coil $\rightarrow$ helix transition took place at DP = 8 [3]. This suggestion is in excellent agreement with literature data [13-15] derived from oligo (γOBzl-L-Glu) prepared by stepwise syntheses. Thus, it is a logical consequence that a polymerization of γOMe-L-Glu-NCA initiated with oligomeric (γOBzl-L-Glu)$_n$ (No.12, Table I) leads to a higher α-helix content than a benzylamine-initiated polymerization under similar conditions (No.3, Table I). In other

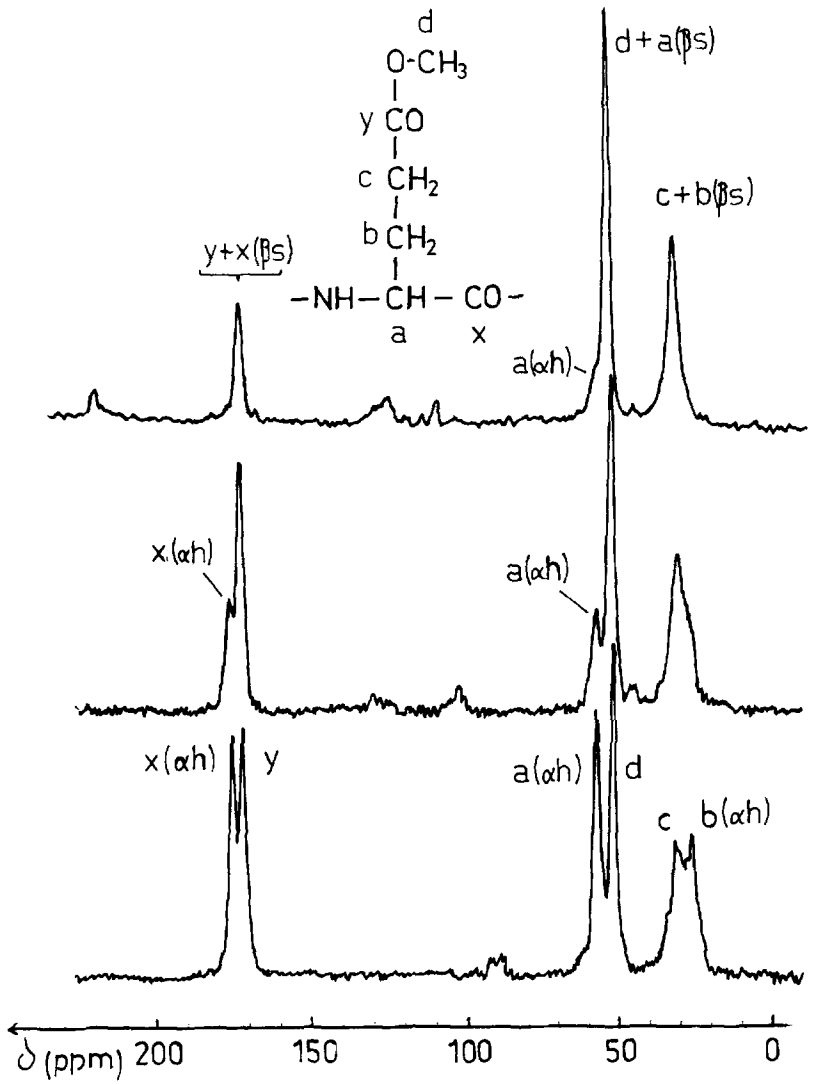

Fig. 1: 50.3 MHz ^{13}C NMR CP/MAS spectra of $(\gamma OMe\text{-}L\text{-}Glu)_n$
A) Sample No. 1, Table I; B) Sample No. 2, Table I
C) Sample No. 10, Table I.

words, oligomeric $(\gamma OBzl\text{-}L\text{-}Glu)_n$ can serve as α-helix inducing initiator for other NCAs.

Table I. Reaction conditions and results of various polymerizations of γOMe-L-Glu-NCA

No	Initiator	Mon/In	Solvent	Temp. (°C)	Time (d)	Yield[a] (%)	α-Helix (%)	$\overline{DP}$ [b]
1	Benzylamine	10:1	Dioxane	20	4	99	15- 25	10 ± 1
2	Benzylamine	20:1	Dioxane	20	4	98	40- 46	19 ± 2
3	Benzylamine	50:1	Dioxane	20	4	88	48- 55	43 ± 3
4	Benzylamine	20:1	Methylenechloride	20	2	98	50- 58	19 ± 2
5	Benzylamine	20:1	Acetonitrile	20	2	99	62- 70	19 ± 2
6	Benzylamine	20:1	Tetrahydrofuran	20	2	100	62- 70	19 ± 2
7	Benzylamine	20:1	Methanol	20	2	100	0(-10)	≤ 12
8	Triethylamine	50:1	Acetonitrile	20	4	88	95-100	---
9	Triethylamine	50:1	Dioxane	20	4	91/95	95-100	---
10	Pyridine	1:25	Pyridine	20	8	96	95-100	---
11	Polyethylene oxide (Mn = 5.000)	20:1	Dioxane	20	4	96	95-100	---
12)	$Ip(\gamma OBzl\text{-}L\text{-}Glu)_{10}$ [c]	20:1	Dioxane	20	4	98	80- 86	---

a) after precipitation from cold diethylether

b) ^1H NMR end-group analyses by means of the aromatic benzyl protons

Table II. Results of isopropylamine-initiated polymerizations of $(\gamma OBzl\text{-}L\text{-}Glu)_n$ in various solvents at 25°C

No	Mon / In	Solvent	Time (d)	Yield (%)	α-Helix (%)	$\overline{DP}$ c)
1	10:1	Ethylacetate	2	93 a)	75- 80	10 ± 1
2	20:1	Ethylacetate	2	95 a)	90- 95	18 ± 1
3	50:1	Ethylacetate	4	94 a)	97-100	47 ± 2
4	10:1	Dioxane	2	94 b)	80- 85	9 ± 1
5	20:1	Dioxane	2	97 b)	97-100	20 ± 2
6	20:1	Methylenechloride	2	98 a)	97-100	20 ± 2
7	20:1	Dimethylformamide	2	94 b)	97-100	19 ± 2

a) precipitated into cold diethylether

b) precipitated into cold water (pH2)

c) ^1H NMR endgroup analyses of isopropylamide CH_3 signals

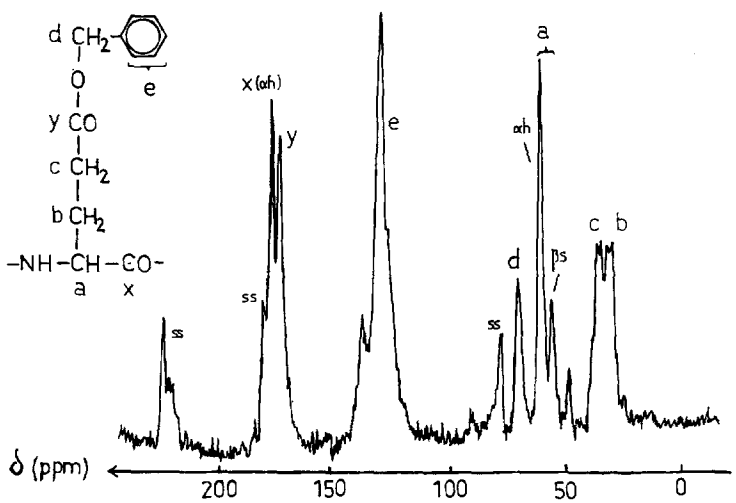

Fig.2 75.4 MHz ^{13}C NMR CP/MAS spectrum of $(\gamma OBzl\text{-}L\text{-}Glu)_n$, No. 1, Table II

EXPERIMENTAL

Syntheses and polymerizations of γOMe-L-Glu-NCA and γOBzl-L-Glu-NCA were conducted as described for Ala- and Leu-NCA previously [3,4]. The NMR measurements were also performed as described previously [2].

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