Analysis

Secondary Structure of Peptides

12. $^{13}$C NMR CP/MAS Study of Amorphous Polypeptides Prepared by Solid State Polymerization of Amino Acid N-Carboxyanhydrides (NCAs)

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SUMMARY

In order to investigate the NMR spectroscopic characteristics of amorphous peptides and proteins, we have attempted to prepare amorphous polypeptides in three ways: first by polymerization of L-(or D-)NCAs in the melt; second by polymerization of L-(or D-)NCAs in the solid state; third by oligomerization of D,L-NCAs in solution or in the solid state. Only the last two methods yielded disordered oligo- and polypeptides. Their $^{13}$C NMR CP/MAS spectra display broad signals with line widths in the range of 5-10 ppm (350-750 Hz); yet their chemical shifts do not differ from those of highly ordered secondary structures. Upon reprecipitation amorphous poly(L-amino acid)s assume highly ordered conformations whereas oligo (D,L-amino acid)s do not significantly change their secondary structure.

INTRODUCTION

In previous papers of this series $^{1-8}$ we have reported that $^{13}$C NMR CP/MAS spectroscopy enables a qualitative and quantitative analysis of the secondary structure of synthetic polypeptides and silk fibroins. It was demonstrated that $\beta$-sheets are detectable in the presence of $\alpha$-helices (or vice versa) $^{1-7}$, $3_{1}$ helices in the presence of $\beta$-sheets $^{2}$ or $10_{3}$ helices (in the case of poly(proline)) $^{2}$ and a $\alpha$-helix in the presence of both $\alpha$-helix and $\beta$-sheet structure $^{8}$.

However, in addition to these highly ordered secondary structures, proteins, and in particular the complex skleroproteins may contain considerably amounts of amorphous regions. Hence, prior to NMR spectroscopic investigations of such proteins the spectroscopic characteristics of amorphous regions need to be studied by means of model peptides.
RESULTS and DISCUSSION

The main difficulty of spectroscopic studies of amorphous peptides is their preparation. Due to hydrogen bonds, strong dipole-dipole interactions and severe steric interactions of the side chains, peptides prefer only two or three energetically favourable conformations as demonstrated by Rachamandran diagrams. Furthermore, the time required for conformational rearrangements of dissolved peptides is only on the order of $10^{-8}$ s. Hence, any synthesis of peptides in solution will yield a highly ordered secondary structure. For the same reasons reprecipitation of amorphous peptides will considerably increase the conformational order. Nonetheless, for two reasons oligo (D,L-amino acid)s with a random sequence of D,L-monomer units are the only exception of this rule. First, polymerization degrees (DPs) below $12 \pm 1$ (depending on the nature of the amino acid) prevent the formation of helices. Second, a random sequence of enantiomers prevents the formation of ordered $\beta$-sheets.

On account of this situation we attempted to prepare sterically uniform (isotactic) polypeptides with a low degree of conformational order by polymerization of L- or D-amino acid NCAs in the melt or in the solid state. When molten L-Phe-NCA, L-$\gamma$-OMe-Glu-NCA and L-Leu-NCA were polymerized at 145-150°C, the resulting polypeptides contain a substantial fraction (up to 20%) of low molecular weight byproducts, which were extracted by means of ethanol. Regardless, whether the polypeptides were extracted or not the NMR spectra displayed the typical signal patterns of polypeptides with a high content (70-90%) of $\alpha$-helix structure. Neither the chemical shifts, nor the line widths differed from those of polymers prepared in solution, and after reprecipitation, the spectra were almost unchanged. Hence, it is obvious that polymerizations of molten L- or D-NCAs do not yield polypeptides with a substantial fraction of amorphous regions.