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A Study of a $C^{\alpha,\beta}$-didehydroalanine Homo-oligopeptide Series in the Solid-state by ^{13}C Cross-polarization Magic Angle Spinning NMR

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Abstract: The fully extended peptide conformation (2.0_5-helix) has been investigated for the first time in the solid-state by ^{13}C cross-polarization magic angle spinning NMR. The compounds examined are members of a terminally protected, homo-oligopeptide series (from monomer through hexamer) based on $C^{\alpha,\beta}$-didehydroalanine.

Keywords: cross-polarization magic angle spinning NMR; $C^{\alpha,\beta}$-didehydroalanine peptides; ^{13}C NMR; peptide secondary structure; solid-state NMR

INTRODUCTION

^{13}C cross-polarization with magic angle sample spinning (CPMAS) is a standard one-dimensional solid-state NMR method for investigating the molecular structure in polycrystalline or powder samples. An isotropic chemical shift spectrum is obtained with this method, which is useful for 3D-structural studies because the ^{13}C isotropic chemical shift is sensitive to backbone torsion angles and hydrogen bonding patterns [1–9]. As a result, CPMAS has been used extensively to investigate the secondary structure of model polypeptides and natural polypeptide fibers [3,9–21]. In addition to backbone conformation, the ^{13}C chemical shift is also sensitive to the presence of charged groups and end effects, resulting in resolution of each residue in short peptide oligomers [11]. In some cases, by taking advantage of the sufficient resolution, oligomers with similar structure but inequivalent positions in the unit cell have been resolved [11].

Cross-polarization (CP) enhances the poor signal intensity of solid-state NMR spectra through transfer of magnetization from protons to nearby ^{13}C nuclei through dipolar interactions. As a result: (i) the extent of polarization enhancement at each site depends on the strength of the $^{1}H-^{13}C$ dipolar interaction, and (ii) CP is not an inherently quantitative technique. However, CPMAS has been well established as an accurate method for quantitatively determining mixtures of backbone conformations present in polypeptide samples [3,9,10,14,20,21].

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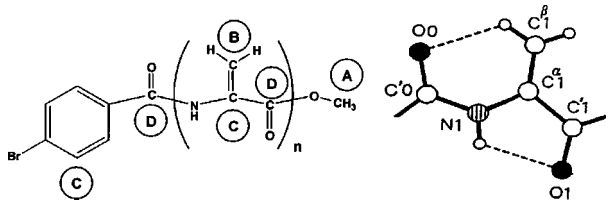
This property is related to the uniform efficiency of CP to a particular type of carbon within a regular peptide structure, so that the number of nuclei contributing to each peak can be determined from the peak area as long as all peaks under comparison correspond to the same type of carbon (i.e. all carbonyl carbons, or all C^{α} carbons, etc.).



A few years ago, the first homo-peptide series, *p*BrBz-$(\Delta Ala)_n$-OMe (*p*BrBz, *para*-bromobenzoyl; ΔAla, $C^{\alpha,\beta}$-didehydroalanine; $n = 1–6$; OMe, methoxy) based on a $C^{\alpha,\beta}$-didehydro-α-amino acid was synthesized to determine the preferred conformation of this residue, characterized by an sp^2 α-carbon atom and the smallest side-chain [22]. To this aim, FT-IR absorption and ^{1}H NMR techniques in solution and x-ray diffraction in the crystal state were used. Our investigation showed that a multiple, consecutive, fully extended conformation (2.0_5-helix) [23] largely predominated in deuterochloroform solution and occurred in the crystal state for the monomer, dimer and trimer as well. These peptide molecules are completely flat, including the amino acid side-chains, and form planar sheets. This novel peptide structure is stabilized by two types of intramolecular H-bonds, $N_i–H\ldots O_i{=}C'_i$ (typical of the 2.0_5-helix) and $C^{\beta}{}_{i+1}–H\ldots O_i{=}C'_i$ (characteristic of ΔAla peptides) (see above). This communication presents ^{13}C CPMAS NMR results on the $(\Delta Ala)_n$ homo-oligopeptides mentioned above with the aim at characterizing for the first time this special type of 2.0_5-helix in the solid state by this emerging physico-chemical technique.

MATERIALS AND METHODS

Synthesis of Peptides

The solution synthesis and full chemical characterization of the *p*BrBz-$(\Delta Ala)_n$-OMe ($n = 1–6$) homo-oligomers have already been reported [22].

Solid-state NMR

^{13}C cross-polarization with magic angle spinning spectra of the peptides in solid powder form were recorded on a Varian/Chemagnetics 400 MHz spectrometer with a ^{13}C frequency of 100.618 MHz and a ^{1}H frequency of 400.13865 MHz. A commercial Varian/Chemagnetics double resonance MAS probe with a 5 mm zirconia MAS rotor was used to acquire the spectra. A cross-polarization pulse sequence with TPPM decoupling during acquisition was used with a ^{1}H $\pi/2$ pulse length of 4.7 μs, 3.5 ms CP contact time, 53 kHz CP power, and 61 kHz proton decoupling power. Adamantane was used to set the parameters and reference the spectra with respect to TMS by setting its peaks at 29.5 and 38.6 ppm. $^{13}C–^{13}C$ coupling is ignored since the natural abundance of ^{13}C is low and there will be very few $^{13}C–^{13}C$ pairs. Spinning sidebands are visible in all the spectra, and are marked with an asterisk. As they occur at intervals of the spinning speed on either side of each peak, a spectrum acquired with 5 kHz MAS will have sidebands spaced by (5000 Hz)/(100 Hz/ppm) = 50 ppm. Also the intensity of the sidebands decreases with increasing spinning speed.

RESULTS AND DISCUSSION

Solid-state NMR spectra of powder samples of the $(\Delta Ala)_n$ homo-peptides, obtained using CPMAS, are shown in Figure 1. The peaks were assigned by comparison with predicted isotropic chemical shifts by means of the Wiley Interscience Spectral Prediction Program (SpecInfo) using the chemical shift prediction rules and spectral database of organic molecules (for the dimer see Table 1). These predictions are for solution NMR, but since these peptides are not likely to change conformation significantly between solution and solid-state environments [22], they should be reasonable. The chemical shift range typical of each type of carbon is the same in solids or in solution, but there may be small variations between the two environments due to small changes in conformation or intermolecular interactions (for example, crystal packing versus solvent interaction). The solution NMR spectrum of the dimer (Table 1) was obtained in $CDCl_3$ with a trace of acetone.

Comparison of the monomer, dimer and trimer solid-state spectra confirms the assignments (Table 2). In each case there is a single peak near 53.5 ppm (**A**) that corresponds to the *C*-terminal — OCH_3 methyl carbon. As the solid-state

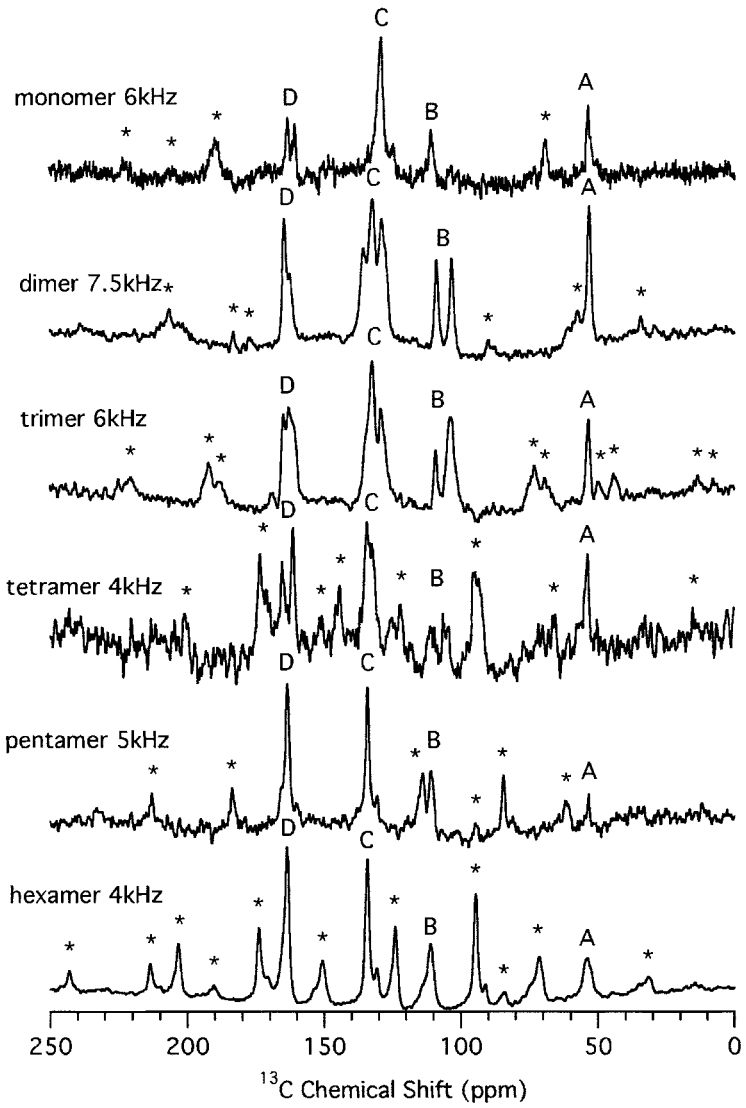


Figure 1 Solid-state ^{13}C CPMAS NMR spectra of *p*BrBz-$(\Delta Ala)_n$-OMe ($n = 1–6$) homo-oligomers. The spectra were obtained at the indicated MAS speeds. Spinning side bands are marked with an asterisk. For the A–D peak designation see text.

NMR spectra were obtained using cross polarization, a comparison of the peak areas to determine the relative number of nuclei is only accurate when all of the peaks correspond to the same type of carbon. Therefore, this analysis is not useful for comparing peaks falling in different regions of a single spectrum, but it is useful for analysing the changes that occur within each region of the spectrum upon addition of another monomer unit.

The peaks labeled **B** correspond to the C^{β} side-chain sp^2 methylene carbons. There is one peak in the monomer, two in the dimer, and two peaks with a 2 : 1 area ratio corresponding to three side-chain carbons in the trimer. Based on this pattern, the side-chain carbon of the residue closest to the *N*-terminus of the molecule has a higher-frequency chemical shift (near 109 ppm), while the side-chain carbons from the other residues have a lower-frequency chemical shift (near 103 ppm). This result is consistent with the x-ray diffraction structures, which showed a difference between the *N*-terminal residue and the rest of the chain in whether or not the oxygen to which the side-chain was $C^{\beta}{}_{i+1}–H\ldots O_i{=}C'_i$ hydrogen bonded shared an additional $N_i–H\ldots O_i{=}C'_i$ hydrogen bond. Based on this pattern, in the tetramer, pentamer and hexamer spectra, two peaks are expected at similar chemical shifts with a ratio of 3 : 1, 4 : 1 and 5 : 1, respectively.

Table 1 Comparison of Predicted and Experimental Chemical Shifts (ppm) for the ΔAla Homo-dimer[a]

Peak	Solid-state chemical shift	Solution chemical shift	Prediction from chemical shift rules	Prediction from spectral database
A (CT)	53.2	53.2	50.5	52.425
B ($C^{\beta}2$)	108.8 (1)[b]	109.6 (1)	103.0 (1)	112 (1)
($C^{\beta}1$)	103.2 (1)	102.5 (1)	103.3 (1)	104.096 (1)
C ($C^{\alpha}1$)[c]	135.4[d]	132.0 (2)	138.8 (1)	133.844 (1)
($C^{\alpha}2$)	132.2	131.7 (2)	138.4 (1)	129.789 (1)
(C4)	128.9	129.0 (1)	132.5 (1)	131.246 (1)
(C2, C6)		128.8 (1)	131.9 (2)	131.308 (2)
(C3, C5)		128.6 (2)	129.5 (2)	129.35 (2)
(C1)			126.5 (1)	128.382 (1)
D (C′2)[d]	164.5 (2)	164.8 (1)	165.0 (1)	163.3 (1)
(C′0)	162.4 (1)	164.2 (1)	164.3 (1)	165.598 (1)
(C′1)		162.7 (1)	162.9 (1)	162.766 (1)

[a] Chemical shift prediction follows simplified rules, which result in fewer unique chemical shifts than observed in the experimental spectra. The chemical shifts of CT, the aromatic ring carbons, C′0 and the carbons of residue 1 ($C^{\alpha}1$, $C^{\beta}1$, C′1) are predicted to be the same in all the oligomers, regardless of length. Thus, the predicted chemical shifts for the monomer are exactly as listed above for the nuclei present in the monomer. The predicted chemical shifts for all residues other than residue 1 are also identical. Thus, the predicted chemical shifts for residues 2–4 in the tetramer are identical to those listed above for residue 2 of the dimer.
[b] The number in parentheses indicates the number of carbon atoms based on the integrated peak area.
[c] Assignments are for predicted chemical shifts. 2D-Experiments would be necessary to assign these peaks in the experimental spectra.
[d] Low resolution (each peak not assignable).

Table 2 Solid-state Chemical Shifts (ppm) for the ΔAla Homo-oligomers

Peak	Monomer	Dimer	Trimer	Tetramer	Pentamer	Hexamer
A (CT)	53.6	53.2	53.5	53.6	53.5	53.6 (s)
B (C^{β})	110.8	108.8 (1)[b]	109.1 (1)	110.5[d]	110.8	110.9 (s)
		103.2 (1)	103.7 (2)	106.4		
				104.3		
C (C^{α} and	132.0[a]	135.4[a]	132.3[a]	134.3[a]	134.0[a]	134.1[a]
aromatics)		132.2	129.2	131.7		
		128.9				
D (C′)	166.2 (1)	164.5 (2)	164.8 (1)	165.3[d]	164.3 (s)	163.6 (s)
	163.5 (1)	162.4 (1)	162.9 (3s)[c]	161.3		

[a] The large number of overlapped peaks from the C^{α} and aromatic carbon atoms prevents a complete assignment.
[b] The number in parentheses indicates the number of carbon atoms based on the integrated peak area.
[c] (s) indicates a pronounced shoulder on the peak which is not fully resolved.
[d] The low intensity of the peaks and overlap with spinning side bands prevents an accurate determination of the peaks and their relative areas.

This is not observed, suggesting that there is a change in internal hydrogen bonding, conformation, or packing so that the chemical environment of the side-chains is altered slightly.

The peaks labeled **C** correspond to the sp^2C^{α} and aromatic ring carbons. For these carbons there is too much overlap clearly to resolve the behaviour of the separate peaks. It is worth pointing out that there is a single major peak in the monomer, pentamer and hexamer, but multiple peaks in the other oligomers, again suggesting that there is a slight difference in conformation or packing of the different members of the series which results in a greater variation of chemical environment in the dimer and trimer.

Peaks **D** correspond to the carbonyl carbons, one in the *N*-terminal cap and one in each residue. There are two peaks in the monomer spectrum and two peaks in a 2 : 1 ratio corresponding to three carbonyls in the dimer spectrum, as expected. There are also two peaks in a 3 : 1 ratio, accounting for the four carbonyls, in the trimer, but there is not a clear pattern that can be used to assign the peaks to the added residues in the longer oligomers. As observed in the region **B** of the spectrum, there is a greater variation in chemical shifts in region **D** for the shorter dimer and trimer than for the longer oligomers. This finding is consistent with what is usually observed for peptides based on coded amino acids. In very short oligomers end effects dominate, since the chemical environment of a carbon in a residue at an end of a peptide is not quite the same as a residue in the middle. As the peptide gets longer, there are many more middle residues than end residues. Consequently, the end effects disappear at about the pentamer length. Since both *N*- and *C*- ends of these peptides are capped in ways that maintain the planar structure and the hydrogen-bonding pattern, it is reasonable that the end effects would diminish between the trimer and pentamer lengths.

In summary, for the first time in the solid-state by ^{13}C CPMAS NMR the fully extended peptide conformation (2.0_5-helix) [23] has been characterized although of a special type (the ΔAla C^{α} and C^{β} atoms are sp^2 hybridized). The present results complement those already reported for the most common conformations (α-helix, β-sheet and 3_{10}-helix) responsible for the 3D-architecture of peptides and proteins. Some discrepancies observed among the spectra of the homo-oligomers may arise from end effects (see above) or from modest irregularities in the solid-state (powder) packing modes and hydrogen-bonding patterns which are not expected to occur in regular single-crystal motifs or in a structure-supporting solvent as $CDCl_3$ [22].

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