

Conformational Behavior of Poly(β -benzyl L-aspartate) in the Solid State As Studied by Variable-Temperature Carbon-13 Cross-Polarization/Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT: Carbon-13 cross-polarization/magic angle spinning nuclear magnetic resonance experiments are carried out for poly(β -benzyl L-aspartate) (PBLA) over a wide range of temperatures, in order to elucidate the stability of the conformation in the solid state. From these experimental results, we obtained the following results on the thermal stability of the main chain. The main chain of PBLA takes on a right-handed α -helical conformation (α_R -helix) within the temperature range from room temperature (23 °C) to 117 °C, and the main-chain conformation predominantly changes at about 128 °C from an α_R -helix form to a left-handed ω -helical form (ω_L -helix), although minor transition to the β -sheet form occurs. In the temperature range from 128 to 139 °C a mixture of three types of conformations, the α_R -helix, ω_L -helix, and β -sheet forms, coexist. At 150 °C the α_R -helix form completely transforms to the ω_L -helix or β -sheet form, and in the temperature range from 150 to 161 °C a mixture of both the forms exists. At 173 °C the main-chain conformation completely becomes the β -sheet conformation.

Introduction

It is well-known that the ^{13}C chemical shifts of individual amino acid residues of a number of polypeptides and natural proteins in the solid state as determined by the cross-polarization/magic angle spinning (CP/MAS) method are significantly displaced depending on their main-chain conformations, as defined by the torsional angles (ϕ and ψ) of the skeletal bonds, such as α -helix, ω -helix, 3_1 -helix, 3_{10} -helix, and β -sheet forms,¹⁻¹¹ and not strongly influenced by a specific amino acid sequence.⁷⁻⁹ This view was supported by the theoretical calculations of the contour map of the ^{13}C chemical shift utilizing the finite perturbation (FPT-INDO) theory¹² and the sum-over-states tight-binding molecular orbital (MO) theory^{13,14} and semiempirical approaches.^{15,16} These approaches permit one to use the conformational-dependent ^{13}C chemical shift as an intrinsic probe to elucidate the conformational features in the solid states as viewed from the individual amino acid residues under consideration. Thus, it is feasible to carry out conformational characterization of polypeptides, if the ^{13}C chemical shifts of suitable reference polypeptides are available.

Although many polypeptides have been investigated to ascertain which mechanism is operative for the characteristic conformation-dependent ^{13}C chemical shifts in the solid state, it is obvious that poly(β -benzyl L-aspartate) (PBLA) provides one excellent source of the experimental data, because this polypeptide has four kinds of polymorphs depending on the conditions of the crystallization: α_R -helix, α_L -helix, ω_L -helix, and β -sheet forms. It was previously reported that ^{13}C chemical shifts of various conformations of PBLA in the solid state were determined by the CP/MAS method at room temperature.¹¹

Furthermore, the α_R -helical PBLA is readily transformed into the ω_L -helix with a change of the helix sense by heating at about 70–140 °C and subsequently, the additional heat treatment will result in the change in

conformation from the ω_L -helix to the β -sheet form. For these samples, it is more effective to utilize the variable-temperature (VT) ^{13}C CP/MAS NMR experiments since one is able to elucidate exactly the structure and dynamics at real temperature.

The purpose of this work is to clarify the conformational behavior of PBLA in the solid state over a wide range of temperatures through the VT ^{13}C CP/MAS NMR experiments.

Experimental Section

Materials. Poly(β -benzyl L-aspartate) (MW = 58 000) was purchased from Sigma Chemical Co. Ltd. A thermolabel sheet used to correct the real temperature of an NMR probe in the NMR experiment, of which the color irreversibly changes with temperature, was purchased from Nichiyugiken Kogyo Co. Ltd.

Measurements. ^{13}C CP/MAS NMR spectra were measured by means of a JEOL-GSX270 NMR (67.5 MHz) with a variable-temperature (VT) CP/MAS accessory in the temperature range from room temperature (23 °C) to 173 °C. The sample (ca. 200 mg) was contained in a cylindrical rotor made of ceramic materials and spun at 3–4 kHz. The contact time was 2 ms, and the repetition time was 5 s. The spectral width and number of data points were 27 kHz and 8K, respectively. The ^1H field strength was 1.6 mT for both the CP and decoupling processes. The number of accumulations was 200–400 to achieve a reasonable signal to noise ratio. ^{13}C chemical shifts were calibrated indirectly through external adamantane (29.5 ppm relative to TMS). Spinning sidebands appearing due to an insufficient spinning rate were removed by total suppression of the sidebands (TOSS) method.¹⁷ The correction for real temperature of an NMR probe was made by using a thermolabel sheet. A lot of thermolabel pieces were placed in an oven with a thermometer and heated slowly at a heating rate of 1 °C/3 min. The relationship with real temperature and the color of the thermolabel pieces was observed in the temperature range 50–200 °C. Then, the correlation for temperature indicated in the NMR instrument was done as below. First, a piece of thermolabel sheet was contained in a cylindrical rotor made of ceramic materials and spun at 3–4 kHz at any temperature. After 30 min, the thermolabel was taken out of the cylindrical rotor, and the colored thermolabel sheet showed real temperature in the NMR probe.

The correction of temperature indicated in the NMR instrument was achieved by using the thermolabel pieces. Figure 1

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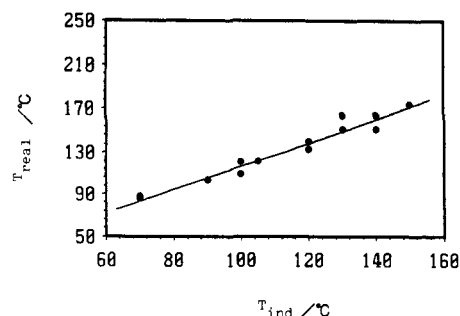


Figure 1. Calibration of real temperature in an NMR probe. T_{real} is the real temperature as determined by the thermolabel sheet method (see the text), and T_{ind} is the temperature indicated in an NMR instrument.

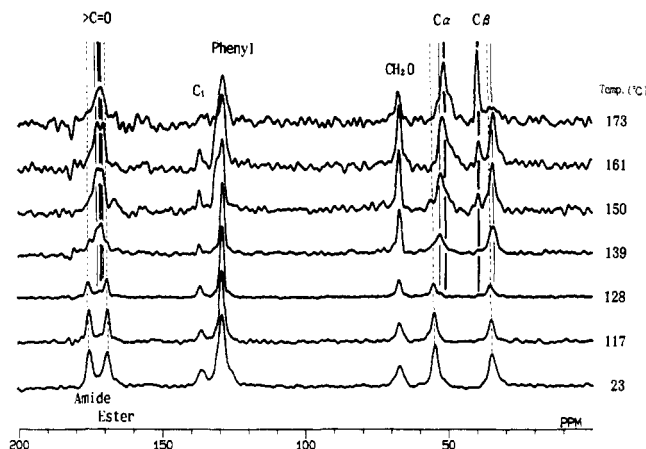


Figure 2. VT ^{13}C CP/MAS TOSS NMR spectrum of poly(β -benzyl L-aspartate) in the solid state at various temperatures: dashed line, α_R -helix form; light solid line, ω_L -helix form; bold solid line, β -sheet form.

shows the correlation between real temperature in an NMR probe and temperature indicated in NMR instrument. The relationship between them is represented by the following equation:

$$T_{\text{real}} = 1.11T_{\text{ind}} + 5.8 (^{\circ}\text{C})$$

where T_{real} is real temperature in an NMR probe and T_{ind} is temperature indicated in an NMR instrument.

Results and Discussion

VT ^{13}C CP/MAS TOSS NMR Spectra of PBLA. The VT ^{13}C CP/MAS TOSS NMR spectra of PBLA in the solid state at temperatures from room temperature to 173 $^{\circ}\text{C}$ were observed as shown in Figure 2, in order to investigate the temperature change of the main-chain conformation of PBLA at various temperatures. The assignment of peaks for the CO (amide), C_α , and C_β carbons in the solid state is straightforward in view of reference data as reported previously.¹⁴ Therefore, the main-chain conformation can be determined from the observation of ^{13}C chemical shift values for PBLA in the solid state by reference data.¹⁴ The obtained ^{13}C chemical shift values of individual carbons in PBLA at various temperatures are listed in Table I. The chemical shift values were determined by computer-fitting.

At room temperature (23 $^{\circ}\text{C}$), ^{13}C chemical shift values of the CO (amide), C_α , and C_β carbons are 175, 55, and 35 ppm, respectively. These values are characteristic of the α_R -helix conformation. In the temperature range from room temperature to 117 $^{\circ}\text{C}$ the major conformation takes the α_R -helix form as seen from Table I. At 128 $^{\circ}\text{C}$ the main-chain conformation is mainly the α_R -helix form, but the small minor peaks which come from the ω_L -helix form appear at 33.6 (C_β) and 52.5 (C_α) ppm, and also the small minor peaks which come from the β -sheet appear at 39.6 (C_β) and 51.4 (C_α) ppm. At 139 $^{\circ}\text{C}$ the CO (amide) peak shifts upfield and the CO (ester) peak shifts downfield, and so the two peaks overlap with each other. Similarly, the C_β and C_α peaks become broad and shift upfield. The chemical shift values of these peaks were determined by computer-fitting. From these results it is found that the conformation is a mixture of the α_R -helix, ω_L -helix, and β -sheet forms. At 150 $^{\circ}\text{C}$ the peaks corresponding to the α_R -helix form completely disappear. At 150 and 161 $^{\circ}\text{C}$ we can recognize two peaks due to C_β carbons at 34.5 and 39.5 ppm. The C_α and the CO (amide and ester) carbon peaks become a broad peak. In this temperature range, it is assumed that the ω_L -helix (34.5 ppm) and β -sheet (39.5 ppm) conformations are mixed. At 173 $^{\circ}\text{C}$ the peaks corresponding to the ω_L -helix conformation completely disappear. The ^{13}C chemical shift values of CO (amide),

Table I
Observed ^{13}C Chemical Shift Values and Conformations of PBLA at Various Temperatures (ppm from TMS)

carbon	^{13}C chemical shift ^a							conformation
	RT	117 $^{\circ}\text{C}$	128 $^{\circ}\text{C}$	139 $^{\circ}\text{C}$	150 $^{\circ}\text{C}$	161 $^{\circ}\text{C}$	173 $^{\circ}\text{C}$	
C_β	34.9	35.3	35.3	35.3				α_R -helix
			33.6	34.2	34.5	34.7		ω_L -helix
			39.3	39.5	39.4	39.5	39.7	β -sheet
			54.8	54.7				α_R -helix
C_α	54.6	54.9	52.5	52.8	52.4	52.0		ω_L -helix
			51.4	51.4	51.2	51.2	51.4	β -sheet
			67.1	66.9				α_R -helix
			sh	67.1	67.1	67.1		ω_L -helix
$\text{CH}_2\text{-O}$	67.2	67.3	sh	67.3	67.3	67.3	67.4	β -sheet
			sh	128.7	128.8	128.8		α_R -helix
			sh	128.7	128.7	128.7	128.8	ω_L -helix
			sh	128.7	130.8	130.8		β -sheet
phenyl	129.2	129.1	129.0	129.0				α_R -helix
			sh	128.7	128.8	128.8		ω_L -helix
			sh	128.7	128.7	128.7	128.8	β -sheet
			sh	128.7	130.8	130.8		ω_L -helix
phenyl (C_1)	136.2	136.3	136.6	136.8				α_R -helix
			sh	137.2	136.9	136.8		ω_L -helix
			sh	136.3	136.3	136.3	136.3	β -sheet
			169.1	169.1				α_R -helix
ester (CO)	169.0	169.0	170.8	171.0	170.4	170.4		ω_L -helix
			171.6	171.6	171.6	171.7	171.6	β -sheet
			175.5	175.1				α_R -helix
			173.0	172.9	172.8	172.9		ω_L -helix
amide	175.1	175.4	171.6	171.6	171.6	171.7	171.6	β -sheet

^a sh = shoulder.

Table II
Determined Relative Peak Intensities of the Carbonyl Carbons (Amide and Ester) and C_β Carbons in PBLA at Various Temperatures

T (°C)	relative peak intensity (%)					
	C_β carbon			carbonyl carbon		
	α_R -helix	ω_L -helix	β -sheet	α_R -helix	ω_L -helix	β -sheet
RT	100.0			100.0		
117	100.0			100.0		
123	76.9	15.4	7.7	78.5	13.6	8.5
139	35.1	56.6	8.4	34.9	55.0	10.2
150		82.4	17.6		80.9	19.1
161		74.9	25.1		77.8	22.3
173			100.0			

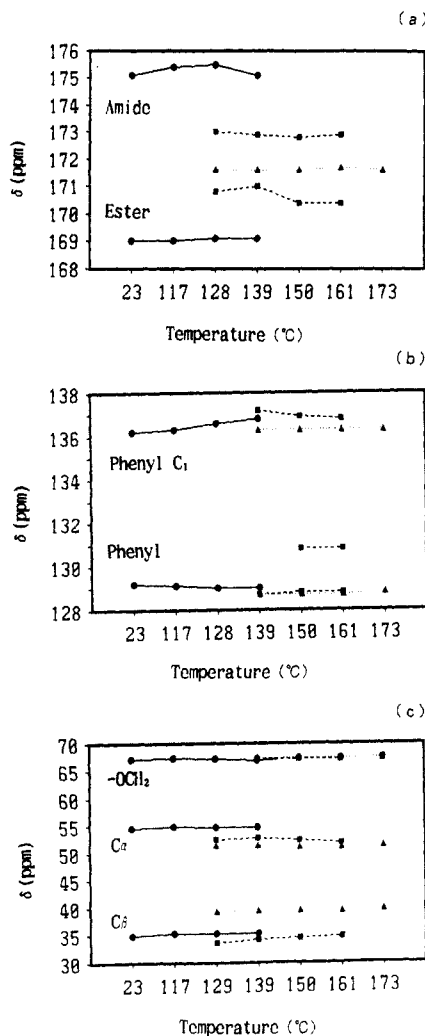


Figure 3. Temperature dependence of ^{13}C chemical shifts for the main-chain carbons and side-chain carbons in poly(β -benzyl L-aspartate) in the solid state: (a) CO (amide and ester), (b) phenyl, (c) $-\text{OCH}_2$, C_α and C_β , (●) α_R -helix, (■) ω_L -helix, (▲) β -sheet.

C_α and C_β carbons are 172, 51, and 40 ppm, respectively. These values are characteristic of the β -sheet conformation. In order to determine the main-chain conformation of PBLA in the solid state, it is very useful to use the ^{13}C chemical shift of the carbonyl carbons (amide and ester) and C_β carbons because they are strongly influenced by the main-chain conformation, as demonstrated previously.¹⁸ The ^{13}C peaks of carbonyl carbons and C_β carbons in the α_R -helix, ω_L -helix, and β -sheet forms appear at 175, 169, and 35; 173, 170, and 35; and 172, 172, and 40 ppm, respectively. These peaks were decomposed by computer-fitting, as reported previously.¹⁸ From these results, the relative intensities of these peaks at various temperatures

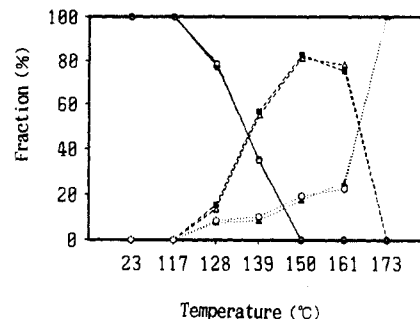


Figure 4. Relative peak intensity of C_β and CO (amide and ester) carbons in the α_R -helix, ω_L -helix, and β -sheet forms as deconvoluted by computer-fitting with Gaussian functions: (●) C_β , α_R -helix, (□) CO, α_R -helix, (■) C_β , ω_L -helix, (▲) CO, ω_L -helix, (▲) C_β , β -sheet, (○) CO, β -sheet.

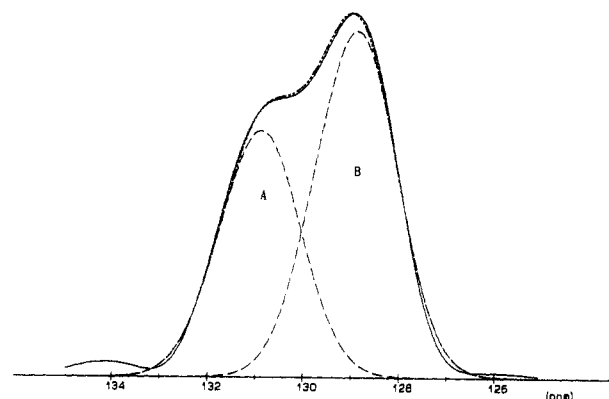


Figure 5. ^{13}C NMR spectra for the phenyl carbon region in poly(β -benzyl L-aspartate) as deconvoluted by computer-fitting with Gaussian functions: (—) observed spectrum, (---) theoretical spectrum, (---) decomposed spectrum.

are determined as shown in Table II and Figure 3. In the temperature range from 23 to 110 °C it can be said that PBLA predominantly takes the α_R -helix form and the structure of the main chain remains unchanged. At 128 °C the α_R -helix is transformed into the ω_L -helix, where the relative peak intensity of the ω_L -helix is $14 \pm 1\%$, and the minor transformation to the β -sheet form occurs and the relative peak intensity is $8 \pm 1\%$. At 139 °C the three types of conformations, α_R -helix, ω_L -helix, and β -sheet coexist. The fractions of the conformations for the α_R -helix, ω_L -helix, and β -sheet are about 36 ± 1 , 56 ± 1 , and $8 \pm 1\%$, respectively.

In the temperature range from 150 to 161 °C the ω_L -helix and β -sheet forms coexist. In this range the fraction of the β -sheet form is gradually increased with an increase of temperature and the relative peak intensity of the ω_L -helix is about 82–75%. On the other hand, the side-chain phenyl carbons peaks appear in the vicinity of about 129 ppm as the two peaks with different chemical shifts (A, 128.8, and B, 130.8 ppm) by the computer-fitting as shown in Figure 4. According to X-ray diffraction studies by Bradbury et al.,^{19,20} in the ω_L -helix form the phenyl rings take two orientations, and then it is expected that two peaks corresponding to two orientations of the phenyl rings are observed. The relative peak intensities of peaks A and B are 41 and 59%, respectively. Peak B may overlap with another peak which comes from the β -sheet form (about 20%). As mentioned above, at 139 °C PBLA partly takes the ω_L -helix (its fraction is about $56 \pm 1\%$). The peaks for phenyl carbons will be expected to be observed like the above case. However, only one peak for the phenyl carbons (about 129 ppm) is observed. From these results, it can be said that at first the main-chain of PBLA changes to the ω_L -helix form from the α_R -helix form in this

temperature range and subsequently the side chains of PBLA rearrange at 150 °C. In the temperature range from room temperature to 161 °C, the helical sense change occurs. By the further heat treatment from 161 to 173 °C the main chain of PBLA with the ω_L -helix form sharply transforms into the β -sheet form.

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Registry No. PBLA (homopolymer), 25248-99-1; PBLA (SRU), 25736-41-8.