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^{13}C CHEMICAL SHIFTS OF SOLID POLYPEPTIDES BY CROSS POLARIZATION/MAGIC ANGLE SPINNING (CP/MAS) NMR SPECTROSCOPY: CONFORMATION-DEPENDENT ^{13}C SHIFTS CHARACTERISTIC OF α-HELIX AND β-SHEET FORMS

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Conformation-dependent ^{13}C chemical shifts of poly(L-valine), poly(L-isoleucine), and poly(L-leucine) of α-helix and β-sheet forms in solid state were measured by cross polarization/magic angle spinning (CP/MAS) NMR spectroscopy. It is found that ^{13}C shifts of C_α, C_β and carbonyl carbons of the first two polypeptides exhibit significant conformation-dependent change, while those of poly(L-leucine) show very little change.

^{13}C NMR spectroscopy has proven to be a very useful tool to probe conformation and dynamics of peptides and proteins in solution.[1,2] Nevertheless, it is still premature to be able to predict how and to what extent ^{13}C chemical shifts of individual amino acid residues are displaced depending on conformational change such as folding or unfolding of proteins, because of very few reference data available to this end. Accordingly , it is obviously invaluable to have reference data of ^{13}C chemical shifts of polypeptides in particular conformations such as α-helix and β-sheet forms. It has been shown by ^{13}C NMR studies of some polypeptides in solution that C_α and C=O signals of α-helix are significantly displaced downfield compared with those of random-coil form,[3] whereas ^{13}C signals of C_β is displaced upfield. Very few data, however, are available for ^{13}C chemical shifts of β-sheet form due to difficulty in recording ordinary high resolution ^{13}C NMR spectra because of low solubility or insolubility in solvent. For this reason, it is useful to record ^{13}C NMR spectra of solid polypeptides by means of newly emerging technique, cross polarization/magic angle spinning (CP/MAS) spectroscopy.[4,5] The most significant advantage to record ^{13}C NMR spectra of solid samples is that ^{13}C chemical shift values free from conformational fluctuation can be obtained for the samples whose conformations are unambiguously determined by X-ray diffraction or other spectroscopic techniques.

Here, we report a ^{13}C CP/MAS NMR study of α-helix and β-sheet polypeptides, poly(L-valine) ($(Val)_n$), poly(L-isoleucine)($(Ile)_n$), and poly(L-leucine)($(Leu)_n$) in solid state, with emphasis on revealing conformation-dependent ^{13}C chemical shifts of polypeptides and comparison of these shifts with ^{13}C shifts recorded in solution state.

Polypeptides in various degrees of polymerization were prepared by polymerizing the N-carboxy-anhydrides of corresponding amino acids in anisole.[6] From infrared, Raman[6] and X-ray diffraction studies,[7] high molecular-weight polypeptides took α-helix, whereas low molecular-weight one or

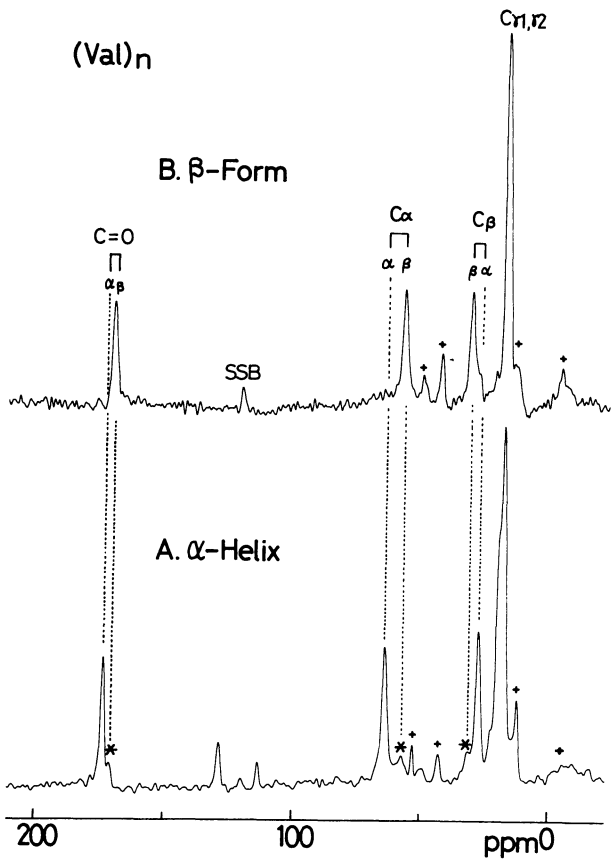

Fig. 1. ^{13}C CP/MAS NMR spectra of solid $(Val)_n$. A. α-helix (1698 transients); B. β-sheet (900 transients).

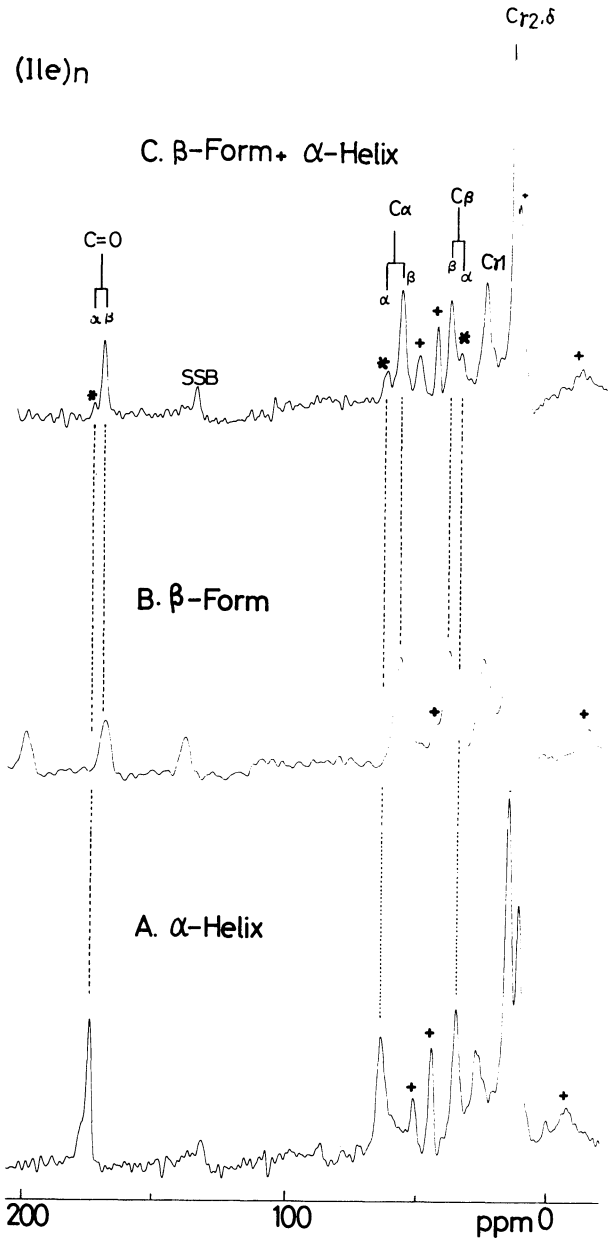

Fig. 2. ^{13}C CP/MAS NMR spectra of $(Ile)_n$ in solid state. A. α-helix (840 transients); B. β-sheet (961 transients); C. β-sheet containing α-helix (1000 transients).

high molecular-weight samples treated with CF_3COOH were β-sheet form. ^{13}C CP/MAS NMR spectra at 75.46 MHz were recorded by a Bruker CXP-300 spectrometer. Contact time and repetition time were 1 ms and 2 s, respectively, with spectral width 30 kHz and 4K data points. Samples (ca. 300 mg) were contained in an Adrew-Beams type rotor machined from perdeuterated poly(methyl methacrylate) and spun as fast as 3-4 kHz. Ordinary high resolution ^{13}C NMR spectra were also recorded by the same spectrometer in CF_3COOD solution.

Figures 1 and 2 show ^{13}C NMR spectra of $(Val)_n$ and $(Ile)_n$, respectively, in solid state. Generally, characteristic peaks of both α-helix and β-sheet forms are well separated, although some peaks at high field region overlap. Peaks marked by + come from the rotor and probe assembly. Peaks from samples are easily assigned, as indicated in the Figures 1 and 2 (α and β stand for α-helix and β-sheet forms, respectively), in the light of high resolution ^{13}C NMR spectra of these polypeptides observed in CF_3COOD solution (spectra not shown) and available ^{13}C shifts data of correspond-

Table 1. Comparison of ^{13}C chemical shifts of α-helix and β-sheet forms of some polypeptides in solid state (ppm from TMS, ± 0.5 ppm)

	$(Val)_n$				$(Ile)_n$				$(Leu)_n$	
	Solid state			Soln.	Solid state			Soln.	Solid state	Soln.
	α-Helix	β-sheet	Δ [a]	Random coil [b]	α-Helix	β-sheet	Δ [a]	Random coil [b]	α-Helix	Random coil [b]
C_α	65.5	58.4	7.1	61.2 (60.1-60.5) [c]	63.9 [d] 62.7 [d]	57.8 [d] 57.3 [d]	6.1 5.4	61.1 (59.5-59.7) [c]	55.7	55.2 (53.6-53.7) [c]
C_β	28.7	32.4	-3.6	31.7 (30.8-31.1) [c]	34.8 [d] 34.8 [d]	39.4 [d] 38.6 [d]	-4.6 -3.8	37.1 (37.4-37.7) [c]	39.5	39.7 (41.1-41.3) [c]
C=O	174.9	171.8	3.1	174.4 (174.8) [c]	174.9 [d] 174.9 [d]	172.7 [d] 171.4 [d]	2.2 3.5	175.8 (174.8) [c]	175.7	177.0 (175.6) [c]
C_γ γ_1	19.0	18.8 [e]		18.0 (18.8-19.3) [c]	27.2 [d] 25.2 [d]	25.9		25.0 (25.4-26.3) [c]	24.1	25.0 (25.0-25.6) [c]
γ_2	20.9	18.8 [e]		18.5 (19.6-20.5) [c]	14.9 [f]	13.7		14.1 (15.8-16.3) [c]		
C_δ δ_1					10.9	g		9.1 (11.5-11.9) [c]	22.5	21.5 (23.0-23.4) [c]
δ_2									20.2	20.2 (21.6-21.9) [c]

[a] Difference of ^{13}C shifts of α-helix with that of β-sheet form. [b] CF_3COOD solution (a few drops of conc. H_2SO_4 were added in the cases of $(Ile)_n$ and $(Leu)_n$). [c] Taken from denatured protein shift range except for C=O peak. Shift values for the latter were from peptide shift range (Ref. 1). [d] ^{13}C shifts data from β-sheet containing α-helix (mixture). [e] Overlapped. [f] Not resolved from the peak at 13.7 ppm. [g] Not resolved from $C_{\gamma 1}$ signal.

ing amino acid residues from a number of denatured proteins and peptides.[1] ^{13}C signals arising from small amount of β-sheet or α-helix forms present with major α-helix or β-sheet conformations (asterisked peaks in Figures 1A and 2C) are also readily identified in view of their peak positions. Those ^{13}C shifts of polypeptides including the data of $(Leu)_n$ are summarized in Table 1, together with the values of random-coil form taken in CF_3COOD solution.

Obviously, ^{13}C signals of the backbone carbons (C_α and C=O)as well as C_β carbons in the side-chain exhibit conformation-dependent change. ^{13}C shifts of carbons in the side-chain other than C_β seem to be rather insensitive to the conformational change and therefore less reliable as a probe to monitor conformational change because of overlapping of signals. Table 1 shows that C_α and C=O resonances of β-sheet form are substantially shifted upfield (5.4-7.1 ppm, and 2.2-3.5 ppm, respectively) with respect to those of α-helix form. On the contrary, C_β signals of β-sheet form are shifted downfield compared with those of α-helix form (-3.6 - -4.6 ppm). This trend is quite reasonable because ^{13}C shifts of backbone carbons are directly related to conformational change, while chemical shifts from carbons at the side-chain (except for C_β) are influenced by conformational change in an indirect manner.

As reference to ^{13}C chemical shift change arising from folding structure of peptides and proteins, displacements of signals from those of random-coil form may be more interesting. Here we use ^{13}C shifts taken in CF_3COOD solution as those of random-coil form. Care must be taken for comparison that carbonyl ^{13}C shifts could be much influenced by ionization, if strong acidic solvents were used. This might happen for $(Ile)_n$ and $(Leu)_n$ because a few drops of conc. H_2SO_4 are added to dissolve samples more easily. In fact, it appears that carbonyl ^{13}C signals of these polypeptides

are resonated downfield compared with the signal of $(Val)_n$. As clearly seen in Table 1, ^{13}C signals (C_α and C_β) of random-coil form appear between the signals of α-helix and β-sheet forms, for $(Val)_n$ and $(Ile)_n$. This result is consistent with a view that chemical shift observed in random-coil form should arise from averaging of ^{13}C shifts of at least these two energetically favored conformations due to rapid chain isomerization in solution. Surprisingly, however, displacements of ^{13}C shifts of α-helical $(Leu)_n$ in solid state are very small compared with those of random-coil form in solution. For understanding the role of the side-chain in stabilizing suitable conformation, a further study on polypeptides with variety of side-chains may be necessary.

As far as ^{13}C chemical shifts of the side-chain are concerned, ^{13}C shifts of carbons at the side-chain other than C_β in solid state are in agreement with those of random-coil form in CF_3COOD solution. There may be slight deviation between ^{13}C chemical shifts of random coil in CF_3COOD and those of denatured proteins (up to 2 ppm). This problem also remains to be clarified.

The present result strongly suggests that significant conformation-dependent ^{13}C shift, useful probe in determining conformation of biopolymers, should arise from carbons at the backbone moiety, if rate of conformational isomerism around the single bond is sufficiently slow compared with NMR time scale as in solid state. In consistent with this view, it was found that ^{13}C shifts of carbons at the glucosidic linkages of polysaccharides are appreciably changed depending on conformations.[8)]

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