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C-13 NUCLEAR MAGNETIC RESONANCE AS A PROBE OF HELIX-COIL TRANSITION OF POLY-L-METHIONINE

Shin-ichi TADOKORO*[1], Shizuo FUJIWARA*, and Yoshihiro ICHIHARA**
* Department of Chemistry, Faculty of Science, The University of Tokyo
Hongo, Tokyo 113
** Central Research Laboratories of Kuraray Co., Kurashiki, Okayama 710

C-13 NMR spectra were measured for poly-L-methionine in $CDCl_3$-trifluoroacetic acid mixtures. The α- and carbonyl carbons showed upfield displacements of 2.3 and 3.4 ppm, respectively, due to the helix-coil transition of the compound. The clearly split peaks assigned to each side-chain carbon of PLM in the α-helix and the random-coil form were observed in the range of the helix-coil transition.

The helix-coil transition of synthetic polypeptides has been extensively studied by NMR technique.[1)] In the present paper C-13 NMR was used to investigate the helix-coil transition of poly-L-methionine (PLM) in the $CDCl_3$-trifluoroacetic acid (TFA) mixtures.

PLM was prepared by polymerization of L-methionine N-carboxyanhydride initiated with triethylamine and its molecular weight was determined as about 2200 by the terminal amino group analysis. A proton NMR study has shown that the helix-coil transition of this sample takes place in $CDCl_3$-TFA(65:35) mixture[2)]. C-13 NMR spectra were obtained at 25.15 MHz on a JEOL PS-100 spectrometer equipped with the PFT-100 Fourier Transform System. The concentration of the sample solution was 20%(W/V) and an 8 mm sample tube was used. The composition of $CDCl_3$-TFA solvents was presented by the volume percent. Chemical shift values (accurate to ±0.12 ppm) are presented in ppm relative to the external standard TMS sealed in a coaxial inner tube. Spin-lattice relaxation times were measured by use of a 180°-t-90° pulse sequence.

Typical C-13 NMR spectrum of PLM is reproduced in Fig. 1, and the chemical shift values are tabulated in Table 1 together with the spin-lattice relaxation times. In the range of the helix-coil transition, two clearly split peaks were observed for

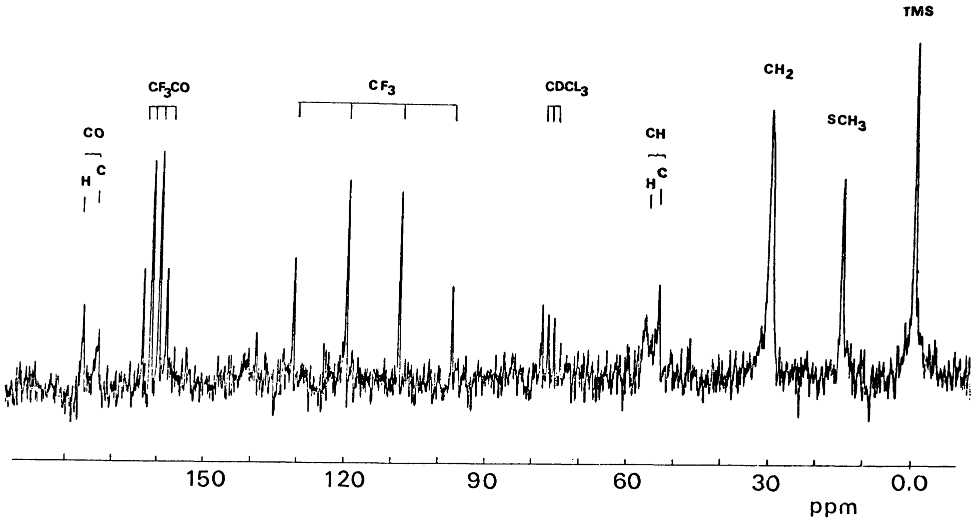

Fig. 1 Proton-decoupled natural-abundance C-13 NMR spectrum of poly-L-methionine (200mg/ml) in CDCl$_3$-TFA (65:35) mixture. Spectrum was recorded at 25.152 MHz, with 8192 points in the time domain and 6250 Hz sweep width.

both the α- and carbonyl carbons (Fig. 1). Lower field peaks are attributed to the helix form and the ones of the upper field to the random-coil form. The differences for the α- and the carbonyl carbon chemical shifts were measured as 2.3 and 3.4 ppm, respectively. These results are consistent with the results of poly-γ-benzyl-L-glutamate$^{3)}$ and poly(N-δ-carbobenzoxy-L-ornithine).$^{4)}$

As in the case of the backbone carbons, the side-chain carbons give multi-peaks in the range of the helix-coil transition (Fig. 2-B). By comparing the spectrum of the sample with those of PLM of α-helix or random-coil form, the sample is assumed to contain both α-helix and random-coil forms. With respect to the peaks at ~30 ppm, the lowest and the highest peaks are attributed to the methylene carbons of PLM in random-coil form, and the intermediate one to the methylene carbons of PLM in the α-helix form. With respect to the peaks at ~15 ppm, the upfield peak is assigned to the methyl carbon in the random-coil form, and the lowfield one to that in the α-helix form. These assignments are consistent with the results of measurements of relaxation times that the peaks assigned to the α-helix carbons present spin-lattice relaxation times shorter than those assigned to the random-coil form.

With respect to the source of chemical shift difference between the α-helix and random-coil carbons, the arguments which have so far been made seem not agreeable after our finding obtained in the study. Those arguments in the literature which

Table 1 Chemical shift values[a)] and spin-lattice relaxation times[b)] on various carbons of poly-L-methionine in $CDCl_3$-TFA mixtures.

$CDCl_3$/TFA	90/10	65/35	35/65
Carbon	(α-helix)	(helix-coil)	(random-coil)
S-CH_3		15.27 (1.06±0.01)	14.80 (0.60)
	16.07 (1.01)	15.49 (0.84±0.03)	
CH_2		30.45 (0.13±0.02)	30.15 (0.12)
	31.06 (0.09)	30.82 (0.09±0.03)	
CH_2		30.99 (0.12±0.02)	30.88 (0.06)
$^{\alpha}CH$		54.23 (0.14)	54.11 (0.08)
	56.78 (0.11)	56.48 (0.11)	
CO		173.94 (0.91)	173.94 (0.90)
	177.22 (0.95)	177.34 (1.18)	

a) Chemical shifts are presented in ppm from external TMS (±0.12 ppm).

b) Spin-lattice relaxation times are presented in parentheses (sec.).

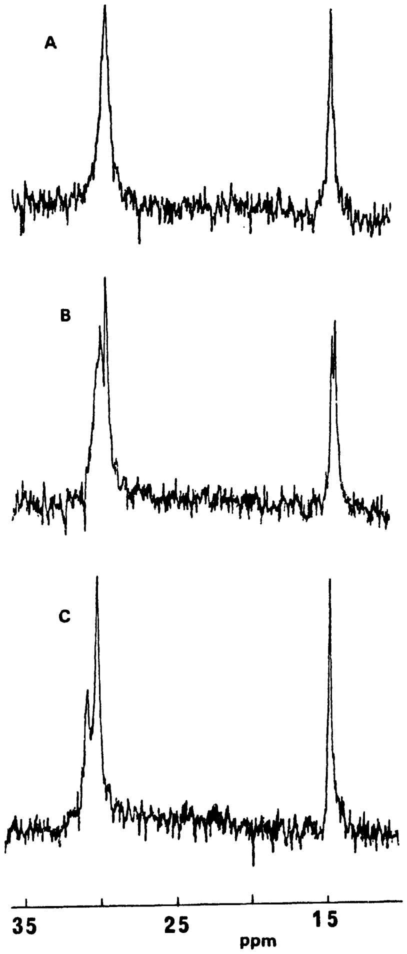

Fig. 2 Proton-decoupled natural-abundance C-13 NMR spectra of poly-L-methionine (200 mg/ml) in $CDCl_3$-TFA mixtures, (A) $CDCl_3$/TFA = 90/10, (B) 65/35 and (C) 35/65. These spectra were recorded at 25.146 MHz, with 8192 points and 2000 Hz sweep widths.

relate the chemical shifts of side-chain carbons presume the effects of solvent.[4)] However, this seems not simply plausible as the sample such as the one adopted in this study which does not carry any polar group in the side-chain presents sizable chemical shift as reported in the text. The present authors observed appreciable difference of the spin-lattice relaxation times between the α-helix and the random-coil carbons in the side-chain skeletons, which may refer to the fact that the chemical shift difference so attributed to the orientation of the side-chain in the α-helix form.

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*1 On leave from Central Research Laboratories of Kuraray Co., Kurashiki, Okayama.

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