Conformational Characterization of Wool Keratin and S-(Carboxymethyl)kerateine in the Solid State by ^{13}C CP/MAS NMR Spectroscopy

H. Yoshimizu and I. Ando*

Department of Polymer Chemistry, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo, Japan

Received November 17, 1989; Revised Manuscript Received December 20, 1989

ABSTRACT: ^{13}C CP/MAS NMR spectra of native wool fiber and four kinds of S-(carboxymethyl)kerateines extracted from wool (low-sulfur fractions (SCMKA), helix-rich fragments (SCMKA-hf), high-sulfur fractions (SCMKB), and high-glycine–tyrosine fractions (HGT)) were measured in the solid state. By use of the observed ^{13}C chemical shift values of the main-chain carbonyl carbons, the major conformations of SCMKA and SCMKA-hf were determined to be the right-handed α-helix form and those of SCMKB and HGT to be the β-sheet form. It was also confirmed that both the right-handed α-helix and β-sheet forms exist in native wool fiber. Further, it was suggested that the coiled-coil structure exists in wool, SCMKA, and SCMKA-hf.

Introduction

It has been demonstrated from experimental and theoretical studies[1-17] that ^{13}C NMR chemical shifts of polypeptides in the solid state determined by the cross-polarization/magic angle spinning (CP/MAS) method are substantially conformation-dependent, depending on their main-chain conformations such as α-helix, ω-helix, 3_1-helix, and β-sheet forms. Furthermore, the side-chain conformations of the L-phenylalanine (Phe) and L-tyrosine (Tyr) residues of oligopeptides or homopolypeptides in the solid state could be determined by means of the ^{13}C CP/MAS NMR method.[18] It appears that the ^{13}C NMR chemical shifts of amino acid residues of polypeptides are not strongly influenced by a specific amino acid sequence, but the conformation-dependent ^{13}C chemical shifts arise mainly from the local conformation of the amino acid residues. Such a conformation-dependent ^{13}C NMR chemical shift may be the most powerful tool for conformational characterization of polypeptides and proteins in the solid state.

The clarification of the fine structure of fibrous proteins in the solid state is very important for the understanding of their natures. So far, structural characterization of silk,[4,5] collagen,[7] elastin,[19] and tropomyosin[20,21] have been carried out by means of ^{13}C CP/MAS NMR, and it was demonstrated that ^{13}C CP/MAS NMR is a very useful means for obtaining information about the secondary structures and the higher order structures. However, according to our best knowledge, there is little systematic ^{13}C CP/MAS NMR information of wool and/or keratin in the solid state except for a ^{13}C CP/MAS NMR study of horse hoof, horse hair, parrot feather, and human hair by Kricheldorf and Müller.[22] The native wool fiber consists of intermediate filaments (termed "microfibrils") composed of low-sulfur proteins which are embedded in a nonfilamentous matrix. The nonfilamentous matrix usually contains two classes of proteins; one is high-sulfur protein and the other is protein containing glycine and tyrosine residues (high-glycine–tyrosine protein).[23] Wool keratin can be divided into three main fractions after reduction of disulfide bonds and protection of the resulting thiol groups with iodoacetic acid to form S-(carboxymethyl)kerateine (SCMK).[24-26] In addition, a helix-rich fragment can be obtained from the low-sulfur keratin fraction of SCMK (SCMKA) after partial hydrolysis with α-chymotrypsin.[27]

In this work, we aim to measure ^{13}C CP/MAS NMR spectra of wool fiber and the four kinds of SCMK in the solid state and to investigate their conformations. A great number of the investigations on the structure of wool and/or SCMK have been performed by means of ORD, IR, CD, X-ray diffraction, and so on, but the conformational characterization of wool is not complete because of its complexity. It is expected that the ^{13}C CP/MAS NMR experiments may complement these prior investigations.

Experimental Section

Materials. Merino 64 wool was repeatedly washed with 0.3% aqueous sodium dodecyl sulfate (SDS) solution, chilled ethanol, and acetone. SCMK was prepared from wool according to the procedure of O'Donnel and Thompson[25] and was fractionated into three fractions; low-sulfur proteins (SCMKA), high-sulfur proteins (SCMKB), and high-glycine–tyrosine proteins (HGT) according to the procedure of Dowling and Crewther.[26] Three fractions were freeze-dried. Helix-rich fragments obtained from SCMKA (SCMKA-hf) were prepared by partial hydrolysis with α-chymotrypsin from bovine pancreas (purchased from Wako Pure Chemical Industries Ltd.) according to the method of Crewther and Dowling.[27] The purification of SCMKA-hf was performed by dialyzing its aqueous sodium tetraborate solution against deionized water. The weight percentages of SCMKA, SCMKB, and HGT from the total amount of SCMK obtained in this preparation were 74.7, 22.5, and 2.8 wt %, respectively. These weight percentages are very similar to the results reported previously.[28] Table I summarizes the amino acid compositions of SCMKA, SCMKA-hf, SCMKB, and HGT determined by a HITACHI L-8500 amino acid analyzer after hydrolysis with 6 N HCl at 110 °C for 20 h, together with those of merino wool reported previously.[29] The contents of the helix-forming amino acid residues such as the L-aspartic acid (Asp), L-glutamic acid (Glu), L-alanine (Ala), and L-leucine (Leu) residues increase, but the helix-breaking amino acid residues such as the L-threonine (Thr), L-serine (Ser), L-proline (Pro), glycine (Gly), and L-cysteine (Cys) residues decrease in the order of wool, SCMKA, and SCMKA-hf. On the other hand, SCMKB contains Thr, Ser, Pro, and Cys residues as its major components, and HGT contains Ser, Gly, Tyr, and Phe residues as its major components. From these findings, it is suggested that the conformational features of wool, SCMKA, SCMKA-hf, SCMKB, and HGT may be different from each other.

Measurements. ^{13}C CP/MAS NMR spectra were recorded on a JNM GSX-270 NMR spectrometer operating at 67.8 MHz with a CP/MAS accessory. Samples (ca. 100–200 mg) were contained in a cylindrical rotor made of zirconia and spun as fast as 4–4.5 kHz. Contact time was 2 ms, and repetition time was

0024-9297/90/2223-2908$02.50/0

Table I
Amino Acid Compositions of Wool, SCMKA, SCMKA-hf, SCMKB, and HGT

	amino acid composition, residues/1000 residues				
amino acid	wool[a]	SCMKA	SCMKA-hf	SCMKB	HGT
Asx[b]	64	85	101	25	34
Thr	65	52	41	107	38
Ser	102	93	70	129	122
Glx[c]	119	148	197	84	4
Pro	59	39	27	123	69
Gly	86	80	39	65	263
Ala	53	63	74	32	21
Val	55	61	60	58	31
1/2 Cys	106				
CM-Cys[d]		60	48	190	50
Met	5	7	3	0	1
Ile	31	36	42	33	2
Leu	77	96	126	36	52
Tyr	40	38	32	24	163
Phe	29	29	20	18	99
Lys	31	32	44	5	1
His	9	8	7	8	7
Arg	68	73	69	63	43

[a] Reference 29. [b] Asx means Asp and Asn. [c] Glx means Glu and Gln. [d] CM-Cys means (carboxymethyl)cysteine.

5 s. Spectral width and data points were 27 kHz and 8 K, respectively. Spectra were usually accumulated 400–2000 times to achieve a resonable signal-to-noise ratio. The ^{13}C NMR chemical shifts were calibrated indirectly through external adamantane (29.5 ppm relative to tetramethylsilane). Spinning sidebands, appearing due to insufficient spinning rate of sample, were removed by the TOSS method[31] except for the dipolar dephasing experiment.[32] In the dipolar dephasing (DDph) experiment, the delay time for proton decoupling and data acquisition was 60 μs, which was long enough to eliminate the ^{13}C signals of all protonated carbons except for methyl carbons.

Results and Discussion

Assignment of ^{13}C Peaks. ^{13}C CP/MAS TOSS NMR spectra of wool, SCMKA, SCMKA-hf, SCMKB, and HGT in the solid state are shown in Figure 1 together with ^{13}C CP/MAS DDph NMR spectra for their aliphatic carbons. The peaks of the carbonyl, aromatic, C_α methine, and side-chain aliphatic carbons appear at about 172–175, 115–158, 45–60, and 10–40 ppm, respectively. The peaks of the Thr, Ala, Val, Leu, and L-isoleucine (Ile) methyl carbons clearly appear at about 10–23 ppm, and, especially, the peak of the Leu C_δ methyl carbons clearly appears at about 23 ppm only in the ^{13}C CP/MAS DDph NMR spectra. The observed ^{13}C chemical shift values in wool, SCMKA, SCMKA-hf, SCMKB, and HGT are summarized in Table II together with the assignments which are made by using reference data with respect to homopolypeptides in the solid state as reported elsewhere.[1-7,12,13,15-17,33]

Main-Chain Conformational Features. a. ^{13}C Signals of Carbonyl Carbons in Wool, SCMKA, SCMKA-hf, SCMKB, and HGT. For characterization of the main-chain conformation of polypeptides and proteins in the solid state, it is very useful to use ^{13}C chemical shift values of the main-chain carbonyl carbons because they are strongly influenced by the conformation of the main chain but not by varieties of amino acids and/or a specific amino acid sequence, as demonstrated previously.[1-17] The ^{13}C peaks of the main-chain carbonyl carbons in the right-handed α-helix (α_R-helix) and β-sheet forms appear at 175.8 ± 0.8 and 170.9 ± 1.2 ppm, respectively.[6,13] The expanded ^{13}C signals in the carbonyl region of wool, SCMKA, SCMKA-hf, SCMKB, and HGT are shown in Figure 2. It can be seen that the carbonyl ^{13}C signal of wool consists of two major peaks and two minor peaks.

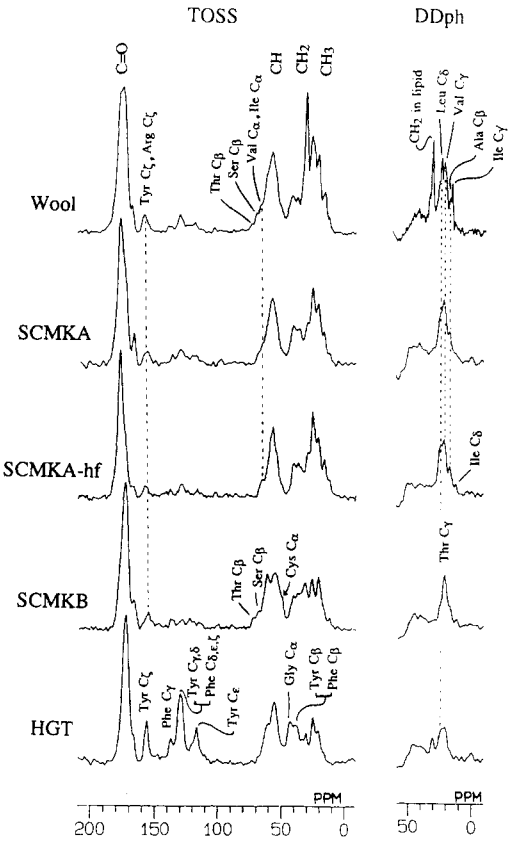

Figure 1. ^{13}C CP/MAS TOSS NMR spectra of wool, SCMKA, SCMKA-hf, SCMKB, and HGT in the solid state and ^{13}C CP/MAS DDph NMR spectra in the aliphatic carbons region.

From the ^{13}C chemical shift values, the major peak at about 176 ppm is assigned to the main-chain carbonyl carbons in the α_R-helix form, and the other major peak at about 172 ppm to the β-sheet form. One minor peak at about 180 ppm probably comes from the side-chain carbonyl carbons of Asp C_γ, Glu C_δ, and carboxymethyl L-cysteine C_ϵ, while the other minor peak at about 166 ppm comes from the NMR rotor made of polyimide. The carbonyl ^{13}C signals of SCMKA and SCMKA-hf are also composed of four peaks, but the relative intensity of the peak appearing at about 172 ppm decreases in the order of wool, SCMKA, and SCMKA-hf. The carbonyl ^{13}C signals of SCMKB and HGT show that the signal appearing at about 172 ppm is an intense single peak. However, the ^{13}C signal of SCMKB is asymmetric with a shoulder at 176 ppm. From these findings, it can be said that SCMKB and HGT are rich in the β-sheet form. The four peaks were decomposed by computer-fitting, and their ^{13}C chemical shift values are about 176, 172, 180, and 166 ppm, respectively. As an example, the observed and deconvoluted ^{13}C signals of the carbonyl carbons of wool are shown in Figure 3. The ^{13}C chemical shift values for the four deconvoluted peaks were determined to be 165.8, 172.3, 176.3, and 180.3 ppm, respectively; half-widths to be 2.7, 4.8, 4.0, and 3.0 ppm, respectively; and relative peak intensities to be 5.0%, 52.0%, 37.4%, and 5.6%, respectively. By use of these results, the relative intensity of the peak at 180.3 ppm was calculated to be 6% (=5.6/(52.0 + 37.4 + 5.6) × 100). Similarly, the relative peak intensities of the side-chain carbonyl carbons (ca. 180 ppm) of SCMKA, SCMKA-hf, SCMKB, and HGT were determined to be 11%, 12%, 11%, and 1.6%, respectively. These values agree roughly with their amino acid compositions determined here, as shown in Table I.

Now, we are concerned with the ^{13}C chemical shift behavior of the main-chain carbonyl carbon for the sam-

Table II
Observed ^{13}C NMR Chemical Shifts for Wool, SCMKA, SCMKA-hf, SCMKB, and HGT in the Solid State

chemical shifts,[a] ppm					
wool	SCMKA	SCMKA-hf	SCMKB	HGT	assignment[b]
175.3	176.0	176.3			carbonyl carbons (αh)
173.2			172.8	172.3	carbonyl carbons (βs)
156.2	155.1	157.2	154.8	156.0 (156.4)	Tyr C_ζ and Arg C_ζ
				136.9 (137.3)	Phe C_γ
128.5	129.1	128.2		128.9 (128.9)	Phe $C_{\delta,\epsilon,\zeta}$ and Tyr C_γ
				116.3	Tyr C_ϵ
72.2			71.8		Thr C_β (βs)
68.6			67.8		Ser C_β (βs)
65.2	c	64.4			Val C_α and Ile C_α (αh)
			60.6		Thr C_α (βs) and Pro C_α
				60.2	d
56.6	56.9	56.4	54.8	54.6	C_α methine carbons
				42.5	Gly C_α
40.5	40.3	40.3	40.1		d
				38.4	Tyr C_β and Phe C_β
36.3	35.6	36.7	36.6		d
30.1 (30.0)					mainly CH_2 in lipid
	28.8	28.9	30.8	30.2	d
25.4	25.0	25.2	25.4	24.6	d
(23.1)	(c)	(23.7)		(23.1)	Leu C_δ
20.3 (20.3)	20.7 (20.9)	21.0 (21.1)		20.6 (20.8)	mainly Val C_γ
			20.3 (20.7)		mainly Thr C_γ (βs)
c (16.5)	16.2 (16.5)	16.1 (16.5)	c		mainly Ala C_β (αh)
14.9 (14.8)					mainly Ile $C_{\gamma'}$
11.9 (11.7)	12.1	12.4 (11.9)	12.1		Ile C_δ

[a] The numbers in the parentheses are chemical shifts of the peaks observed in ^{13}C CP/MAS DDph spectra. [b] The assignment was made by reference data of homopolypeptides in the solid state (refs 1–13, 33). αh and βs in the parentheses mean the α_R-helix and β-sheet forms, respectively. [c] Observed as the shoulder peak. [d] Unassigned at this stage.

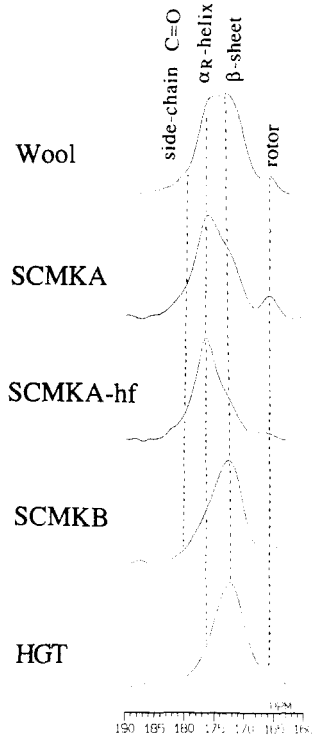

Figure 2. Expanded ^{13}C CP/MAS TOSS NMR spectra for the carbonyl carbon region in wool, SCMKA, SCMKA-hf, SCMKB, and HGT.

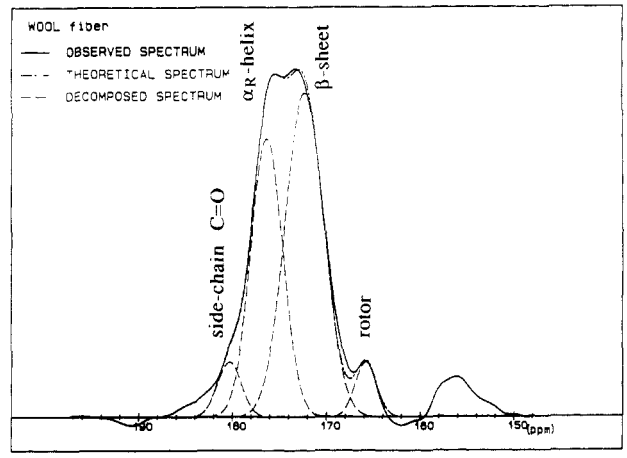

Figure 3. ^{13}C NMR spectra for the carbonyl carbon region in wool deconvoluted by computer fitting with Gaussian functions.

ples considered here in order to discuss the IR conformational features. The carbonyl carbon ^{13}C signal mainly consists of two peaks. Table III summarizes the ^{13}C chemical shifts, half-widths, and relative peak intensities of the main-chain carbonyl carbon in wool, SCMKA, SCMKA-hf, SCMKB, and HGT determined by the computer fitting. (For example, the relative intensities of the two peaks at 172.3 and 176.3 ppm in wool were calculated to be 58% (=52.0/(52.0 + 37.4) × 100) and 42% (=100 − 58), respectively.) The relative intensity of the low-field peak at ca. 176 ppm corresponds to the proportions of the α-helix component because this peak comes from the main-chain carbonyl carbons in the α_R-helix form as mentioned above. From ^{13}C CP/MAS measurements of equimolar mixtures of α-helical poly(L-alanine) ($(Ala)_n$) with poly(glycine) and poly(L-valine),[34] the proportion of the α-helix form obtained by comparison of the peak intensities in $(Ala)_n$ agrees with that obtained by infrared and X-ray diffraction data,[2] so a quantitative evaluation of the secondary structure is feasible. The proportion of the α-helix component increases in the order wool, SCMKA, and SCMKA-hf. On the other hand, the peak at 172 ppm is appreciably upfield from the main-chain carbonyl carbons in the β-sheet form, as mentioned above. The experimental fact that the half-width of the higher field peak is broader than that of the low-field peak can be explained by the previous studies,[6,13] where the range of ^{13}C chemical shifts for the main-chain carbonyl car-

Table III
Observed ^{13}C NMR Chemical Shifts, Half-Widths, and Relative Peak Intensities of the Main-Chain Carbonyl Carbons in Wool, SCMKA, SCMKA-hf, SCMKB, and HGT[a]

	^{13}C chemical shift, ppm	half-width, ppm	relative peak intensity, %
wool	176.3	4.0	42
	172.2	4.8	58
SCMKA	176.2	4.0	56
	172.5	4.8	44
SCMKA-hf	176.4	3.6	65
	173.0	4.5	35
SCMKB	176.0	3.7	25
	172.5	4.6	75
HGT	176.6	4.0	8
	172.2	5.6	92

[a] Determined by computer fitting.

bons in the β-sheet form (170.9 ± 1.2 ppm) was wider than those observed in the α_R-helix form (175.8 ± 0.8 ppm). From the conformational characterization based on the above assignment, it can be said that SCMKB and HGT are rich in the β-sheet form.

b. SCMKB and HGT. SCMKB and HGT are believed to originate chiefly from a nonfilamentous matrix between the microfibrils of the wool fiber, and no evidence has yet been obtained for the existence of any ordered structure in solution or in the solid state.[23] However, it has been recently reported that the existence of the β-sheet form in both SCMKB and HGT films cast from a formic acid solution was identified by means of X-ray diffraction and CD.[35,36] This is also supported by the results obtained here. Further, in the ^{13}C CP/MAS TOSS NMR spectrum of SCMKB (Figure 1), the peaks assignable to the Thr and Ser C_β carbons in the β-sheet form appear at 71.8 and 67.8 ppm, respectively. This implies that the β-sheet form exists to a large extent in SCMKB (Table II). A shoulder peak appearing at about 52 ppm can be assigned to the Cys C_α carbon in the β-sheet form.[37] These results support that in SCMKB the β-sheet form exists to a large extent.

On the other hand, the half-width of the higher field peak (172.2 ppm) of HGT is relatively broader than that of other samples (Table III). This implies that several kinds of conformations besides the α_R-helix and β-sheet forms also exist in HGT. Although the proportion of HGT residues that are Ser is almost the same as that of SCMKB (Table I), in the ^{13}C CP/MAS TOSS NMR spectrum of HGT no peak appears at 65–68 ppm. This implies that the proportion of Ser residues in HGT in the β-sheet form is less than that of SCMKB in the β-sheet form. It has been reported that ^{13}C peaks of the Ser C_β carbons of silk fibroin samples in the silk I form (loose helix[38]) appear at 59.0–61.5 ppm.[5] The peak appearing at 60.2 ppm in the ^{13}C CP/MAS TOSS NMR spectrum of HGT may come from the Ser C_β carbons in a silk I like form. Similarly, the ^{13}C chemical shift value (38.4 ppm) of the peak assignable to the Tyr and Phe C_β carbons[39] is in between the values corresponding to the α_R-helix and β-sheet forms. This implies that the Tyr and Phe residues of HGT reside in conformations other than the α_R-helix and β-sheet forms.

c. SCMKA and SCMKA-hf. SCMKA was considered to originate chiefly from the microfibrils,[23] and the proportion of the α-helix form in aqueous solution was determined to be about 50% by means of ORD[40] and CD.[41] Also, the proportion of the α-helix form of SCMKA-hf in aqueous solution was determined to be about 84% and 90% by means of ORD[42] and CD,[41] respectively. Thus, it can be said that the proportion of the α-helix form in SCMKA-hf is higher than that in SCMKA.

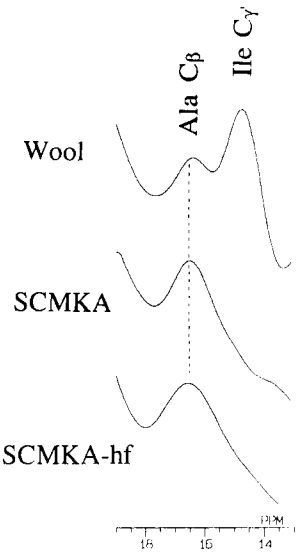

Figure 4. Expanded ^{13}C CP/MAS DDph NMR spectra for the methyl carbon region in wool, SCMKA, and SCMKA-hf.

The same situation can be found in the solid state, as shown in Table III. Moreover, ^{13}C chemical shift values of Val and Ile C_α and Ala C_β carbons in SCMKA and SCMKA-hf suggest that they are rich in the α_R-helix structure (Figure 1 and Table II).

d. Wool. The proportion of the α-helix form in wool was reported to be 30–36% by deuterium-exchange experiments.[43] The carbonyl ^{13}C signal of wool splits into two peak. From their chemical shift values, it is shown that both the α_R-helix and β-sheet forms exist in wool (Figure 2 and Table III). In addition, the ^{13}C CP/MAS NMR spectrum of wool exhibits characteristics of both the reduced and separated matrix and microfibril proteins (Figure 1). The ^{13}C peaks assignable to the Thr and Ser C_β carbons in the β-sheet form appear at 72.2 and 68.6 ppm, respectively, as they do in SCMKB. The ^{13}C peaks assignable to the Val and Ile C_α and Ala C_β carbons in the α_R-helix form appear at 65.2 and 16.5 ppm, respectively, as they do in SCMKA and SCMKA-hf (Table II).

The intensity of the peak appearing at 30 ppm in the spectrum of wool is very strong, but in the spectra of the other samples the corresponding peak is not identified. In general, wool contains lipids of 2.5 wt % in the wool cell membrane. In the lipids, *n*-alkyl methylene (CH_2) carbons undergo fast transition between trans and gauche isomers at room temperature. This situation is very similar to the case of polypeptides having long *n*-alkyl CH_2 side chains[44] or long alkene CH_2 side chains,[45] in which the methylene peak appears at about 30 ppm. From this experimental finding, the peak appearing at 30 ppm in wool may be assigned to the CH_2 carbons of lipids in wool cell membrane. As shown in the ^{13}C CP/MAS DDph experiments, the spin–spin relaxation time (T_2) of the corresponding carbons is relatively long compared with the time of other carbons. This implies that the mobility of its carbons is high. In SCMK samples, lipid components are removed, and so its peak is not identified. These results support the assignment of the peak appearing at 30 ppm in wool to the lipids in the wool cell membrane.

Coiled-Coil Structure of Wool Keratin. The microfibrils of wool are composed of protofibrils which consist of coiled-coil α-helix ropes.[23] The sequence for seven amino acid residues which take the coiled-coil α-helix[23] is found in SCMKA (especially in SCMKA-hf), as well as in tropomyosin, myosin, and paramyosin. Fig-

ure 4 shows the expanded ^{13}C CP/MAS DDph spectra of the Ala C_β carbons in wool, SCMKA, and SCMKA-hf. The ^{13}C chemical shift values of the peaks for the Ala C_β carbons in SCMKA and SCMKA-hf are 16.5 ppm (Table II). In the spectrum of wool, however, two peaks are observed at 14.8 and 16.5 ppm. The ^{13}C chemical shift value of 14.8 ppm is in agreement with that of C_γ methyl carbons of poly(L-isoleucine), and the peak intensity is more intense than that in the ^{13}C CP/MAS TOSS spectrum of wool (Figure 1). This implies that the mobility of its carbons is high. The peak appearing at 16.5 ppm in wool agrees with that in SCMKA and SCMKA-hf. Thus, it can be said that the peaks at 16.5 and 14.8 ppm mainly come from the methyl carbons in the α_R helical and nonhelical forms of wool, respectively. These ^{13}C chemical shift values obviously differ from those of $(Ala)_n$ in the α_R-helix form (15.7 ppm).[46] In our previous study,[21] the two peaks at 15.8 and 16.7 ppm observed in ^{13}C CP/MAS spectrum of tropomyosin in the solid state could be assigned to Ala C_β carbons in the external and internal sites of the coiled-coil structure, respectively, from the difference in the mobility of these carbons. Therefore, ^{13}C chemical shift values of Ala C_β carbons peaks observed in wool, SCMKA, and SCKMA-hf suggest that a number of the Ala residues in these samples are located in the internal site of the coiled-coil structure.

Conclusion

On the basis of the observed ^{13}C chemical shift values of the main-chain carbonyl carbons in SCMKA and native wool fiber samples, the major conformation of SCMKA and SCMKA-hf was determined to be the α_R-helix form, while that of SCMKB and HGT was determined to be the β-sheet form. On the other hand, it is confirmed that both the α_R-helix and β-sheet forms exist in native wool fiber. Therefore, it can be said that the main-chain conformation of matrix proteins is the β-sheet structure, in spite of modifications such as reduction and carboxymethylation of the Cys side chain, as is found for native wool fiber. Further, it is suggested that the coiled-coil structure exists in microfibril proteins.

References and Notes

(1) Taki, T.; Yamashita, S.; Satoh, M.; Shibata, A.; Yamashita, T.; Tabeta, R.; Saito, H. *Chem. Lett.* **1981**, 1803.
(2) Saito, H.; Tabeta, R.; Shoji, A.; Ozaki, T.; Ando, I. *Macromolecules* **1983**, *16*, 1050.
(3) Saito, H.; Tabeta, R.; Ando, I.; Ozaki, T.; Shoji, A. *Chem. Lett.* **1983**, 1437.
(4) Saito, H.; Iwanaga, Y.; Tabeta, R.; Asakura, T. *Chem. Lett.* **1983**, 427.
(5) Saito, H.; Tabeta, R.; Asakura, T.; Iwanaga, Y.; Soji, A.; Ozaki, T.; Ando, I. *Macromolecules* **1984**, *17*, 1405.
(6) Shoji, A.; Ozaki, T.; Saito, H.; Tabeta, R.; Ando, I. *Macromolecules* **1984**, *17*, 1472.
(7) Saito, H.; Tabeta, R.; Shoji, A.; Ozaki, T.; Ando, I.; Miyata, T. *Biopolymers* **1984**, *23*, 2279.
(8) Yamanobe, T.; Ando, I.; Saito, H.; Tabeta, R.; Shoji, A.; Ozaki, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 23.
(9) Yamanobe, T.; Ando, I.; Saito, H.; Tabeta, R.; Shoji, A.; Ozaki, T. *Chem. Phys.* **1985**, *99*, 259.
(10) Ando, S.; Yamanobe, T.; Ando, I.; Shoji, A.; Ozaki, T.; Tabeta, R.; Saito, H. *J. Am. Chem. Soc.* **1985**, *107*, 7648.
(11) Tuzi, S.; Komoto, T.; Ando, I.; Saito, H.; Shoji, A.; Ozaki, T. *Biopolymers* **1987**, *26*, 1983.
(12) Miyamoto, H.; Takezaki, R.; Komoto, T.; Ando, I. *J. Mol. Struct.* **1988**, *172*, 395.
(13) Saito, H.; Ando, I. *Annu. Rep. NMR Spectrosc.* **1989**, *21*, 209.
(14) Ando, S.; Matsumoto, K.; Ando, I.; Shoji, A.; Ozaki, T. *J. Mol. Struct.* **1989**, *212*, 123.
(15) Kricheldorf, H. R.; Mutter, M.; Mazer, F.; Müller, D.; Forster, D. *Biopolymers* **1983**, *22*, 1357.
(16) Kricheldorf, H. R.; Müller, D. *Macromolecules* **1983**, *16*, 615.
(17) Kricheldorf, H. R.; Müller, D.; Ziegler, K. *Polym. Bull.* **1983**, *9*, 284.
(18) Miyamoto, H.; Komoto, T.; Kurosu, H.; Ando, I.; Ozaki, T.; Shoji, A. *J. Mol. Struct.* In press.
(19) Kricheldorf, H. R.; Müller, D. *Int. J. Biol. Macromolecules* **1984**, *6*, 145.
(20) Tuzi, S.; Ando, I. *J. Mol. Struct.* **1989**, *196*, 317.
(21) Tuzi, S.; Sakamaki, S.; Ando, I. *J. Mol. Struct.* In press.
(22) Kricheldorf, H. R.; Müller, D. *Colloid Polym. Sci.* **1984**, *262*, 856.
(23) For example: (a) Fraser, R. D.; MacRae, T. P.; Rogers, G. E. *Keratins: Their Composition, Structure and Biosynthesis*; Charles C. Thomas: Springfield, IL, 1972. (b) Bradbury, J. H. *Adv. Protein Chem.* **1973**, *27*, 111. (c) Fraser, R. D.; MacRae, T. P. *Conformation in Fibrous Proteins*; Academic Press: New York, 1973; Chapter 16.
(24) The term "kerateine" is generally used for the reduced form of keratin.
(25) O'Donnel, I. J.; Thompson, E. O. P. *Aust. J. Biol. Sci.* **1964**, *17*, 973.
(26) Dowling, L. M.; Crewther, W. G. *Prep. Biochem.* **1974**, *4*, 203.
(27) Crewther, W. G.; Dowling, L. M. *Appl. Polym. Symp.* **1971**, *18*, 1.
(28) Fraser, R. D.; MacRae, T. P. *Conformation in Fibrous Proteins*; Academic Press, New York, 1973; p 513.
(29) Bradbury, J. H.; Leeder, J. D. *Aust. J. Biol. Sci.* **1970**, *23*, 843.
(30) For SCMKA: Crewther, W. G.; Harrap, B. S. *Nature* **1965**, *207*, 295. For SCMKA-hf: ref 26. For SCMKB: Lindley, H.; Broad, A.; Damaglou, A. P.; Darskus, R. L.; Elleman, T. C.; Gillespie, J. M.; Moore, C. H. *Appl. Polym. Symp.* **1971**, *18*, 21. For HGT: Gillespie, J. M.; Frenkel, M. J. *Aust. J. Biol. Sci.* **1974**, *27*, 617.
(31) Dixon, W. T.; Shaefer, J.; Sefcik, M. D.; Stejskal, E. O.; Mckay, R. A. *J. Magn. Reson.* **1982**, *49*, 341.
(32) Opella, S. J.; Frey, M. H. *J. Am. Chem. Soc.* **1979**, *101*, 5854.
(33) The ^{13}C chemical shift values of the Thr residue were referred to the data of dipeptides, Gly-L-Thr·$2H_2O$ and Gly-DL-Thr·H_2O, whose complete crystalline structure were determined from X-ray diffraction by: Yadava, V. S.; Padmanabhan, V. M. *Acta Crystallogr.* **1973**, *B29*, 854. Swaminathan, P. *Acta Crystallogr.* **1975**, *B31*, 1608.
(34) Müller, D.; Kricheldorf, R. H. *Polym. Bull.* **1981**, *6*, 101.
(35) Amiya, T.; Miyamoto, T.; Inagaki, H. *Biopolymers* **1980**, *19*, 1093.
(36) Amiya, T.; Kawaguchi, A.; Miyamoto, T.; Inagaki, H. *J. Soc. Fiber Sci. Technol. Jpn.* **1980**, *36*, 479.
(37) The ^{13}C chemical shift values for the C_α carbons of poly(S-Bzl-L-Cys) and poly(S-CBZ-L-Cys) in the β-sheet form were 52.8 and 52.6 ppm, respectively, as reported by Kricheldorf and Müller (ref 16), and the present authors (unpublished data), respectively.
(38) Asakura, T.; Yamaguchi, T. *Nippon Sanshigaku Zasshi* **1987**, *56*, 300.
(39) The amino acid residues which have carbons in the ^{13}C chemical shift value range 35–40 ppm are the Asp, Glu, Val, Cys, Ile, Leu, Tyr, Phe, and Arg residues (refs 6, 13, and 16). However, the proportion of the Tyr and Phe residues in HGT is much larger than in the other residues (Table I). Thus, it can be said that the intensity of the peak at 38.4 ppm mainly comes from the Tyr and Phe C_β carbons.
(40) Harrap, B. S. *Aust. J. Biol. Sci.* **1963**, *16*, 231.
(41) Amiya, T.; Kajiwara, K.; Miyamoto, T.; Inagaki, H. *Int. J. Biol. Macromol.* **1982**, *4*, 165.
(42) Crewther, W. G.; Harrap, B. S. *J. Biol. Chem.* **1967**, *242*, 4310.
(43) Fraser, R. D.; MacRae, T. P.; Rogers, G. E. *Keratins: Their Composition, Structure and Biosynthesis*; Charles C. Thomas: Springfield, IL, 1972; Chapter 7, pp 138–9.
(44) Yamanobe, T.; Tsukahara, M.; Komoto, T.; Watanabe, J.; Ando, I.; Uematsu, I.; Deguchi, K.; Fujito, T.; Imanari, M. *Macromolecules* **1988**, *21*, 48.
(45) Mohanty, B.; Komoto, T.; Watanabe, J.; Ando, I.; Shiibashi, T. *Macromolecules* **1989**, *22*, 4451.
(46) The ^{13}C chemical shift value (15.7 ppm) for the Ala C_β carbons in the α_R-helix form is inferred from ref 21.