A Carbon-13 CPMAS Solid State NMR Spectroscopic Study of Wool: Effects of Heat and Chrome Mordanting

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ABSTRACT

Carbon-13 CPMAS solid state NMR spectroscopy has been used to investigate the effect of heat on wool. Increases in the unsaturation of the keratin are observed at 225°C. The technique has also been used to show that paramagnetic chromium appears to interact uniformly throughout the bulk of the wool fiber when the fiber is treated with a dichromate solution.

Proton nuclear magnetic resonance spectroscopy (NMR) has been widely used to study proteins and provide information about their structure. Proton NMR studies of wool have been confined to the water soluble protein extract and the effect of urea and trifluoroacetic acid on the protein conformation [3, 5, 6].

The fibrous protein silk has been studied in the solid state using carbon-13 cross polarization magic angle spinning (^{13}C-CPMAS) NMR; both the native and denatured forms of the fibroin were examined [1, 21]. Wool is relatively more complex in composition and structure than silk, but we have attempted to extend the technique to investigate the thermal degradation of the protein and the effect of binding paramagnetic chromium with the keratin. We chose the chromium ion because of its industrial use in chrome dyeing processes [8, 9].

Experimental

WOOL TREATMENTS

Merino wool (64s quality) fibers were cleaned by anhydrous extraction with t-butanol and n-heptane [12]. The wool was heated in an oven under atmospheric conditions at 150°C, 170°C, 200°C, and 225°C for specified periods. It was treated in a solution (wool : liquor ratio of 1:20) of potassium dichromate (1.5% on weight of wool (oww)), adjusted to pH 3.5 with formic acid. The wool was boiled in the solution for 30 minutes, then thoroughly washed with deionized water and dried.

NMR METHODOLOGY

NMR spectra were obtained with a JEOL FX-60 QS spectrometer[2] operating at a ^{13}C frequency of 15 MHz. The ^{1}H decoupling ratio-frequency irradiation field strength was 11 gauss. A spectral width of 8000 Hz and a sampling of 2K data points zero-filled to 4K were used for spectral acquisition. Recycle times were 1.5 seconds between pulses to insure full relaxation and response during each transient collected. All chemical shifts were assigned relative to the hexamethylbenzene shift of 17.63 ppm for its CH_3 resonance based on the position of tetramethylsilane. Samples were spun at a rate of approximately 2.4 kHz at the magic angle of 54.7°C in Kel-F bullet-type rotors. No spinning sidebands were apparent. Peak areas were measured by cutting out the appropriate ^{13}C resonance areas and averaging at least four separate weighings.

RELAXATION MEASUREMENTS

Peak height measurements were used to calculate relaxation times ($T_{1\rho H}$ and T_{CH}). Each frequency domain spectrum was obtained with 40 Hz of computer line broadening. Values of T_{CH} (carbon-proton relaxation time) and $T_{1\rho H}$ (spin-lattice relaxation time) were calculated according to literature methods by measuring ^{13}C magnetization as a function of contact times [16]. A double exponential fit was used to analyze the proton $T_{1\rho H}$ and T_{CH} values. The uncertainty in the calculated values was generally ±10%. We also conducted interrupted ^{13}C—^{1}H decoupling experiments, designed to accentuate nonprotonated ^{13}C resonances

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[2] Reference to a brand or firm name does not constitute endorsement by the USDA over others of a similar nature not mentioned.

[19]. A 40 μs delay without proton decoupling was inserted into the pulse sequence prior to data acquisition.

Results and Discussion

To maximize the absolute ^{13}C responses of all the components in the wool, we studied the contact time versus intensity. The resulting plot (Figure 1) shows a typical profile with an initial increase in ^{13}C signal intensity, followed by a decrease as the contact time is increased. The increase in ^{13}C signal amplitude depends on the carbon-proton cross polarization rate T_{CH}; the decline is due to ^{1}H spin-lattice relaxation in the rotating frame $T_{1\rho H}$. The optimum contact time for wool was ~0.5 ms.

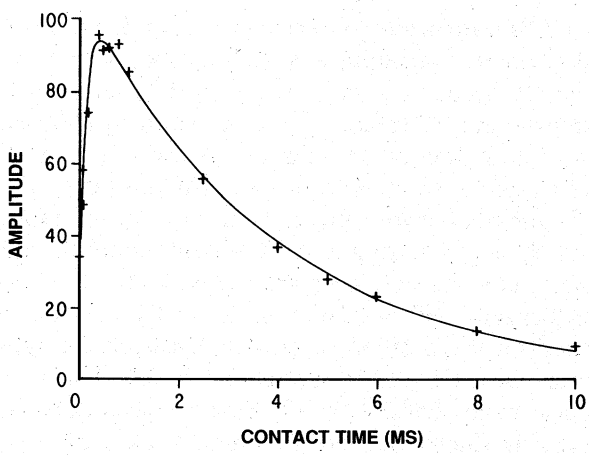

FIGURE 1. Plot of ^{13}C CPMAS relative peak intensities versus contact time (ms) for untreated wool (25 ppm resonance).

Wool is essentially proteinaceous in nature and is composed of twenty amino acids [14]. The ^{13}C CPMAS spectrum of wool (Figure 2, curve A) can be resolved into four distinct, broad peaks and reflects the complex chemical structure of the fiber. The peaks are attributable [23] to a variety of carbon species (Table I), but cannot be specifically assigned to individual amino acids.

TABLE I. Assignments of peaks in the ^{13}C CPMAS spectrum of wool.

Resonance peak position, ppm		Carbon species
25	aliphatic	$\underline{C}H_{2,3}-C$,
55	aliphatic	$\underline{C}H_{1,2}-X$ (X = N, S, O)
130	aromatic	$\underline{C}=C$
175	carbonyl	$\underline{C}=O$

By introducing a 40 μs delay without proton decoupling into the pulse sequence, the nonprotonated carbon population can be differentiated from the protonated carbons. Examination of the interrupted $^{1}H-^{13}C$ decoupled spectrum (Figure 2, curve B) shows that the peak at 55 ppm has disappeared. The major peak in the spectrum is the carbonyl peak centered at 17 ppm, with the aromatic and aliphatic carbon signals, at 130 and 25 ppm, respectively, reduced in intensity. Some methyl group resonances (25 ppm) remain in the spectrum because of the reduced C—H dipolar broadening due to the methyl groups rotational motion. Thus, interrupted decoupling permits the observation and resolution of ^{13}C resonances that are present in relatively low concentrations in the wool system, but which may be critical to the understanding of the structure and composition of wool after chemical modification.

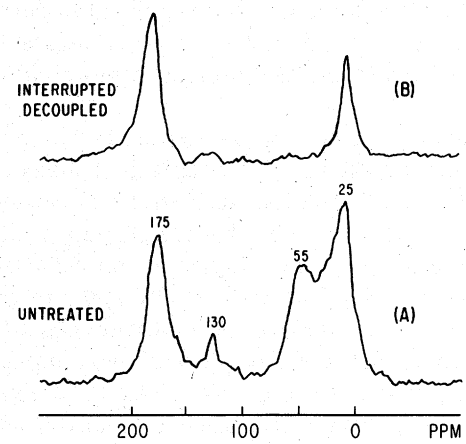

FIGURE 2. (A) ^{13}C CPMAS spectra of untreated wool, (B) same as (A) but with interrupted decoupling.

EFFECT OF HEAT ON WOOL

Heating wool at 150 to 200°C in air for 30 minutes to 4 hours causes an obvious discoloration, but without any discernible change in the ^{13}C NMR spectrum. Heating at 225°C produces and increase in the peak intensity at 130 ppm, however, indicating an increase in the C=C content of the keratin with increasing time (Figure 3).

The increased C=C content may be due to the formation of aromatic species or to dehydrogenation of aliphatic carbons. The resonance positions of aromatic and aliphatic unsaturated carbons overlap and differentiation is difficult. The proportion of each carbon species, shown in Table II, indicates that with increasing heating time, the C=C component increases about

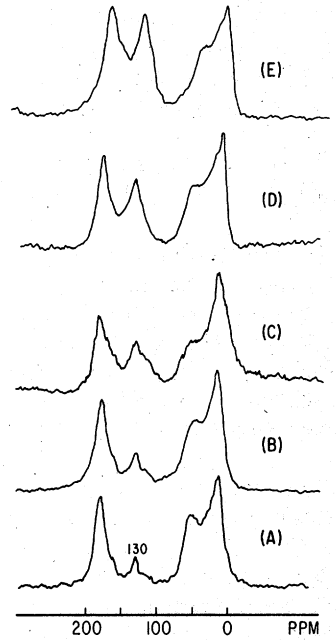

FIGURE 3. Effect of increasing heating time at 225°C on the ^{13}C CPMAS spectrum of wool: (A) original wool, (B) heated for 0.5 hour, (C) heated for 4 hours, (D) heated for 16 hours, (E) heated for 24 hours.

fivefold with a concomitant decrease in aliphatic carbons. The C=O content remains fairly constant throughout the heating; there is, however, an initial decrease, suggesting that the wool is being decarboxylated with the evolution of carbon dioxide.

TABLE II. ^{13}C CPMAS peak area data of wool heated at 225°C.

Length of heat treatment, hours	Resonance peak position, ppm		
	175 (C=O)	130 (C=C)	55 + 25 (aliphatic C)
–	29	4	67
0.5	23	11	67
4	27	19	54
16	31	17	52
24	33	22	45

Heating wool above 150°C is reported to cause a loss of gaseous volatiles [11], amide crosslinking [2], and the formation of new amino acids [17, 18]. Marshall *et al.* [15] showed that wool begins to suffer thermal damage above 200°C. Their findings are consistent with our results. Infrared studies [10] of wool heated to 400°C suggest that C=C bonds are formed. In our ^{13}C CPMAS study, it is evident that the formation of unsaturated carbons occurs at much lower temperatures.

EFFECT OF CHROMIUM ON WOOL

Paramagnetic ions have been used in ^{13}C NMR studies of biological materials to investigate structure [22] and metal binding sites [20] and to improve the quality of the spectra obtained [3, 7]. Previous studies have established that the dichromate (Cr(VI)) absorbed by the wool is reduced to CR(III) upon heating, and that the wool cystine residues are oxidized to cysteic acid [8, 9]. In this preliminary study, we examined the effect of chrome mordanting on the wool fiber using ^{13}C CPMAS NMR spectroscopy.

The ^{13}C CPMAS spectrum of Cr(III)-mordanted wool is no different from that of the untreated sample except for a decrease in the signal-to-noise ratio. The incorporation of chromium into the keratin structure produces a small change in the T_{CH} value (carbon-proton relaxation time) with the $T_{1\rho H}$ value (spin-lattice relaxation time) remaining fairly constant (Table III).

TABLE III. Relaxation values for untreated and chrome-mordanted wool.

Resonance peak position, ppm	$T_{1\rho H}$[a]	T_{CH}[b]
Untreated wool		
175 (C=O)	2.99	0.319
55 (CH_n-X)	3.51	0.089
25 ($CH_{2,3}-C$)	3.78	0.123
Chrome-mordanted wool[c]		
175 (C=O)	3.63	0.775
55 (CH_n-X)	5.01	0.239
25 ($CH_{2,3}-C$)	4.53	0.331

[a] ms ± 10%. [b] ms ± 25%. [c] Chromium content of about 1% oww.

The lack of change in the $T_{1\rho H}$ values shows that proton spin diffusion is uniform throughout the wool, and that discrete binding of the chromium to wool could not be detected. Furthermore, the presence of chromium renders many of the carbons undetectable under our experimental conditions.

The small increase in the T_{CH} value may be due to the shortening of the $T_{1\rho H}$ of protons in the immediate vicinity of chromium, so that $T_{CH} \gg T_{1\rho H}$. Alternatively, the resonance frequency of the protons may become so broad in the presence of the chromium that these protons cannot be adequately irradiated by the short pulse used in this study.

The chromium therefore appears to be distributed uniformly throughout the bulk of the wool fiber, or at least appears to be interacting homogeneously throughout the system. Scanning electron microscopic

studies with x-ray microanalytical systems have shown that in chrome-mordanted wool, the metal ion is deposited evenly throughout the bulk of the fiber [13].

Conclusions

Solid state ^{13}C CPMAS NMR spectroscopy provides a direct, nondestructive probe for evaluating the changes that occur upon heating wool. Increases in the unsaturation of aromaticity of wool occur at about 225°C. The technique also has shown that in chrome-mordanted wool, the metal is distributed such that proton spin diffusion and relaxation values associated with the various functional groups constituting the wool fiber do not diverge significantly. Therefore, no discrete metal binding was detectable under the experimental conditions used.

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