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Primary structure of archaeological silk and ancient climate

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The fractions of glycine residues were determined with the aid of solid-state ^{13}C high-resolution nuclear magnetic resonance for silks found in the coffins of three lords of the Fujiwara clans (12th century in north-eastern Japan). Two indices defined by Nunome were also determined for these samples from electron microscope observation. These three quantities are non-linearly dependent on time (years), and they can be correlated with the temperature (climate) which was determined from dendrochronological data. Copyright © 1996 Elsevier Science Ltd.

(Keywords: archaeology; silk; glycine residue)

INTRODUCTION

Nuclear magnetic resonance has been widely accepted as an excellent characterization method in physics, chemistry, polymer science, and biology, as well as for medical diagnosis. In this paper is shown the applicability of n.m.r. to archaeological silk, i.e. the clarification of the correlation between the primary structure of archaeological silk and the atmospheric temperature when the silk was produced.

In Hiraizumi, a city in north-eastern Japan, there was a feudal government governed by the Fujiwara clan in the 12th century. In the precincts of Chusonji Temple of Hiraizumi, which was founded by Kiyohira Fujiwara, the first lord, there is (still exists) a Konjikido, a hall foiled with gold. Mummies of three lords are still preserved under the floor of this hall. The three lords are: Kiyohira Fujiwara (died in 1128); Motohira Fujiwara (died in 1157); and Hidehira Fujiwara (died in 1187). The fourth lord, Yasuhira Fujiwara, was killed in another area by the army of Yoritomo Minamoto, the lord of another feudal government situated in Kamakura, and the Hiraizumi government fell. This means that the mummies of all of the lords are still preserved. In these mummies many silk materials were used for clothes, winding fabrics, and pillows.

We are interested in the n.m.r. characterization of archaeological silk, as an extension of a long programme of n.m.r. characterization of polymeric materials, since 1959[1] by one (R.C.) of the present authors. At the present stage, the oldest silk sample in Japan is the plain-weave silk attached to a thin-type bronze *ko* excavated from the Arima remains[2]. It is estimated to the production of the Early Yayoi Period (2nd century B.C.). The amount of this silk is quite insufficient for n.m.r. observation. The Shosoin Treasure-House is famous for its collection of silk artifacts from the Nara Period (8th century). These national treasures are impossible to perform n.m.r. observation on. N.m.r. is generally appreciated as a non-destructive analysis. From the standpoint of chemical analysis this is true, but from the standpoint of preservation of national treasures it is not true. Actually, before and after the measurements samples may be identical with each other on the molecular level, but not identical on the materials level. Cutting samples with scissors for n.m.r. measurements is a typical destructive procedure on the latter level. Comparing the samples from the 12th century with those from the 2nd century B.C. and the 8th century, the former are adequate for n.m.r. measurements; the amount is sufficient, and the silk was already broken, on the occasion of tearing from the mummies.

The object of this paper is clarification of the factors influencing the secondary structure of these archaeological silks. We observed solid-state ^{13}C high-resolution n.m.r. of these materials in order to determine the mole fraction of glycine residues. Electron microscopic observations are also performed for the same samples, in order to determine the two indices[2] adopted in the study of archaeological silk; these are the cross-section of the filament and its circularity coefficient. The latter is defined by the ratio of the cross-section of the filament to $(\pi/4)$ (the maximum diameter of the filament)^2. The ancient climate is estimated from the temperature index of each year determined from the data[3] on annual rings of trees.

EXPERIMENTAL

All the samples were taken from the coffins of the three lords. Four samples were used for each clan, taken from different clothes, or from different parts of the same

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clothes. They were a generous gift from Drs W. Kawanobe and C. Sano, Tokyo National Cultural Properties Research Institute. Characteristics of these samples are listed in *Table 1*. They were used for measurements without any pretreatment, in order to avoid further damage to the aged samples.

Table 1 Characteristics of samples used in this paper

Lord	Sample No.	Characteristics
Kiyohira		
	HK 0	Plain weave silk, trousers
	HK 1	Plain weave silk, thin
	HK 2	Plain weave silk
	HK 3	Plain weave silk
Motohira		
	HM 0	Plain weave silk, pillow
	HM 1	Plain weave silk, thick
	HM 2	Plain weave silk, thin
	HM 3	Plain weave silk, corted to the inside of coffin
Hidehira		
	HH 0	Plain weave silk
	HH 1	Plain weave silk, thick
	HH 2	Plain weave silk
	HH 3	Aya cloth

N.m.r. measurements were carried out using a JNM-A 500 spectrometer (125 MHz for ^{13}C nuclei), with CP (Cross Polarization)/MAS (Magic Angle Spinning) mode (spinning frequency of 5.8–6.1 kHz). Spectral deconvolution was achieved with the aid of the deconvolution software, NM-ACFT, installed in the spectrometer.

Microscopic observations were performed using a JEM-2000 EX scanning electron microscope (SEM) (acceleration voltage 200 kV). Before observation the sample was moulded in epoxy resin (Epol 812).

RESULTS AND DISCUSSION

Figure 1 shows the solid-state ^{13}C high-resolution CP/MAS n.m.r. spectra of fabrics winding the mummy of Kiyohira (HK 0), Motohira (HM 0), and Hidehira (HH 0). The peaks appearing at 170–174 ppm have been assigned to C = O of the peptide bonds in fibroin molecules. Splittings in this region are due to the differences in constituent amino acid residues (alanine,

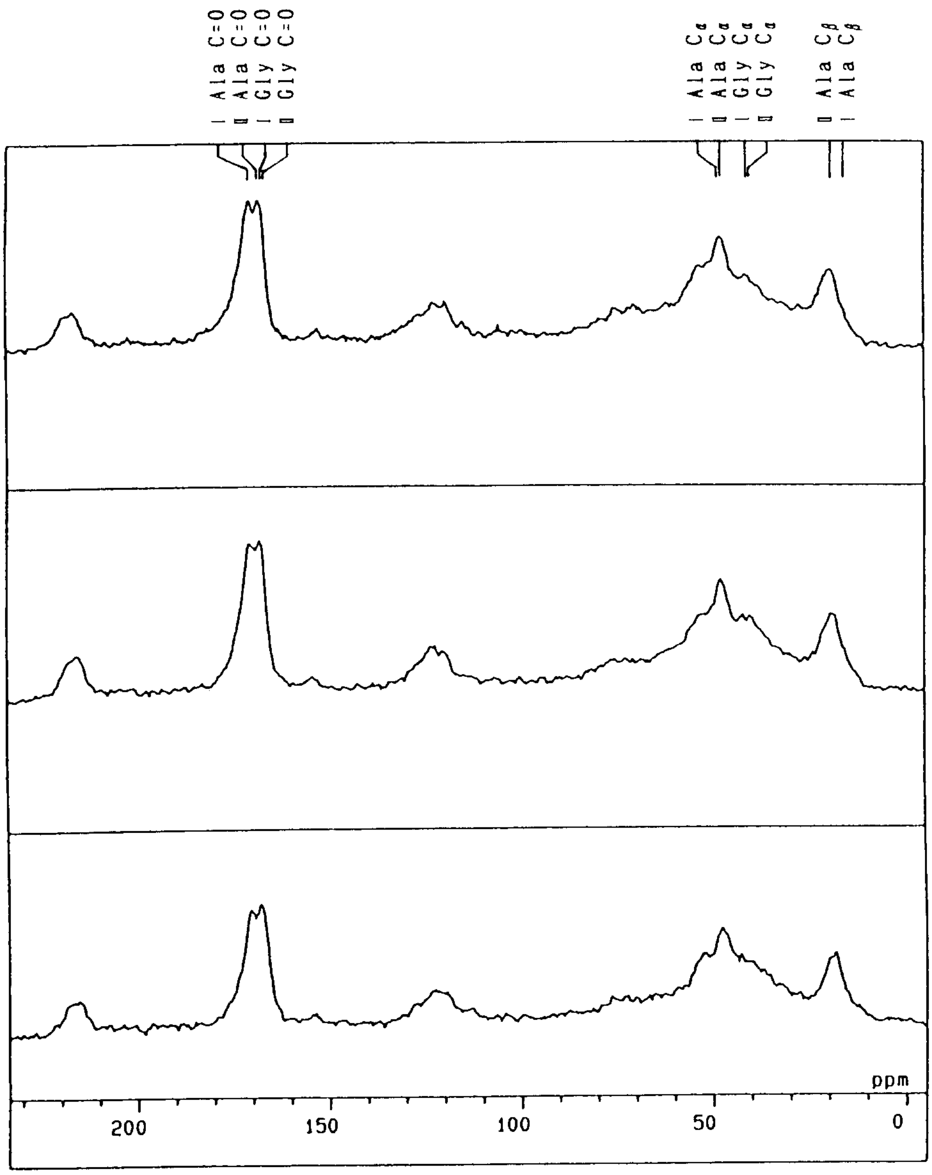

Figure 1 Solid-state ^{13}C high-resolution CP/MAS n.m.r. spectra of fabrics winding the mummy of Kiyohira (HK 0, top), Motohira (HM 0, middle), and Hidehira (HH 0, bottom)

Table 2 The fraction of glycine residues and other parameters of the silks

Lord	Fraction	Cross-section	Circularity coefficient
Kiyohira	46.9 ± 1.1 (%)	49.7 ± 8.4 (μm^2)	54.6 ± 5.0(%)
Motohira	57.2 ± 5.3	40.9 ± 8.4	53.1 ± 4.4
Hidehira	48.6 ± 1.1	72.5 ± 6.6	57.5 ± 1.3

glycine, etc.), as well as those of secondary structure. The former is more significant than the latter[4]. Chemical shift values are 177.0, 172.3, 170.7, and 169.5 ppm from tetramethyl silane for alanine in Silk I, alanine in Silk II, glycine in Silk I, and glycine in Silk II, respectively for fibroin from *Bombix mori*. Silk I is close to random-coil, and Silk II is essentially anti-parallel β-sheet[5]. As a first approximation, the C = O peaks observed were deconvoluted into two parts, namely, the lower and the higher halves. The fraction of glycine residues was calculated from the intensity ratio of the higher field peak to the sum of the lower and higher field peaks. Similar calculations were done for all samples. In the second column of *Table 2* are shown the average values for the four samples, with deviation (maximum not standard). A non-linear dependence of the fraction on time (year) was found. The differences in the fractions between generations are larger than deviations within the same generation. The above dependence is, therefore, clear evidence. The change of fraction is, however, not recognized by silk scientists. It will be confirmed with other methods in future by the present authors.

For archaeological silks there are excellent studies carried out by Nunome[6]. In his studies two important indices have been introduced, i.e. the cross-section and the circularity coefficient, as defined in the Introduction. Both indices have been determined for the present samples from microscopic observation, shown in *Figure 2*. Results of these are also compiled in the third and fourth columns of *Table 2*. From *Table 2* we can find the increase of the fraction of glycine residues is accompanied by the decrease of the other two indices. In other words, there may be a common factor affecting all three of these parameters. Nunome[6] has found good correlation between atmospheric temperature when silk was produced and the two indices introduced by him. In his study he used archaeological data on temperatures in China, Iceland, and other foreign countries. On the other hand, Nara National Cultural Properties Research Institute[7] has established the dendrochronology in Japan. Researchers in that Institute have measured the widths of annual rings in *sugi*, Japanese cedar trees, both for still-living trees and trees used for wooden architecture. They have found better correlation between the widths and amounts of rainfall for trees in the lower latitude area, and better correlation between the widths and temperatures in the higher latitude area. Only graphical data have been published in the report[7]. We therefore asked Dr Mitsutani, one of the authors of reference 7, to give us digital dendrochronological data[3] for trees in the higher latitude area, Iwate. In *Figure 3* is plotted the data supplied by Dr Mitsutani, on widths against years during the 12th century. We can find negative correlation between the fraction of glycine residues and the widths (correlation coefficient = −0.59). We have no information whether the silk was produced in the year of the lord's death, or in the previous year. Fortunately, in the report[6] mutual correlation coefficients have been evaluated between the widths and the temperatures of the corresponding year, of one year previous, two years previous, and so on up to nine years previous. It partly means we can estimate the temperatures from the data of the widths of the corresponding year, of the next year, and so on. Taking into account the coefficients up to five years previous, estimated temperatures (correlation coefficient = −0.95) become almost

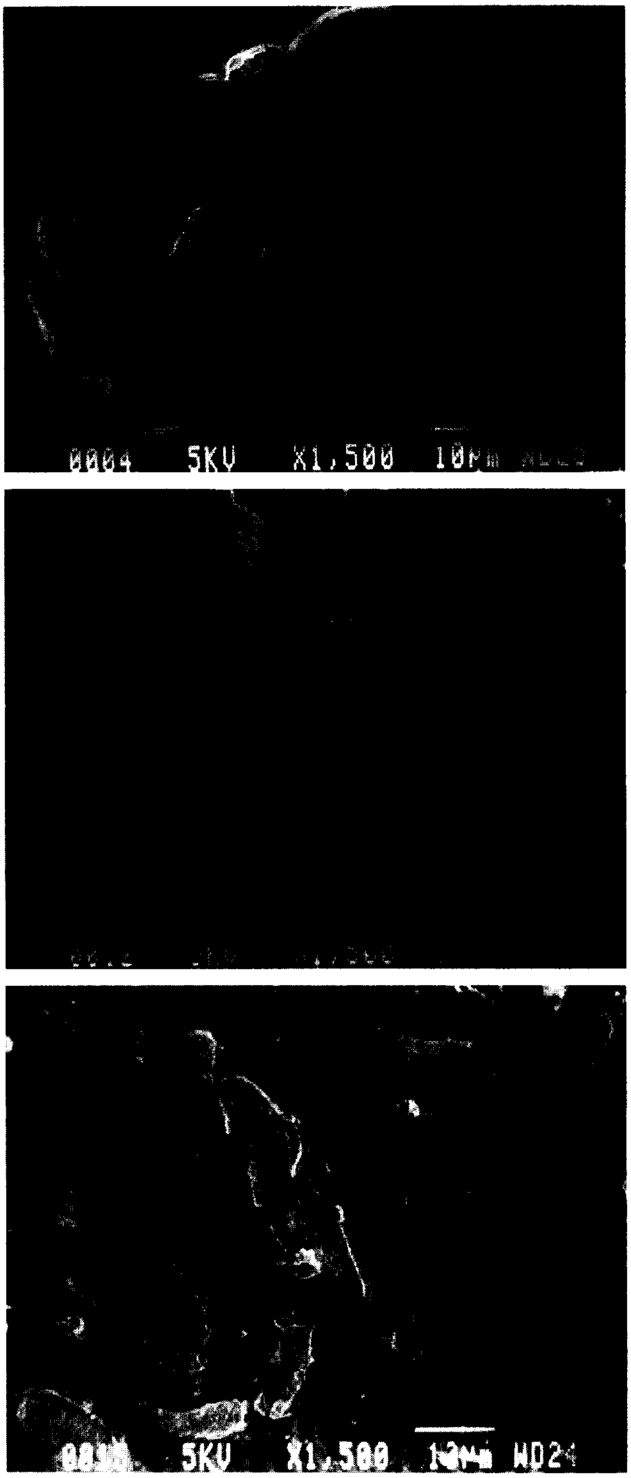

Figure 2 Electron microscope of the cross-sections fabrics winding the mummy of Kiyohira (HK 0, top), Motohira (HM 0, middle), and Hidehira (HH 0, bottom)

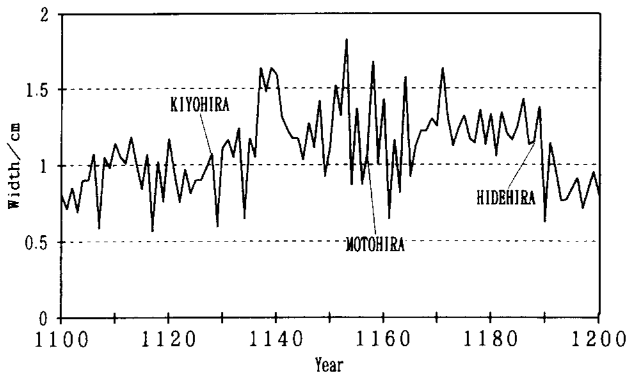

Figure 3 Width of annual rings of *sugi* in Japan during the 12th century

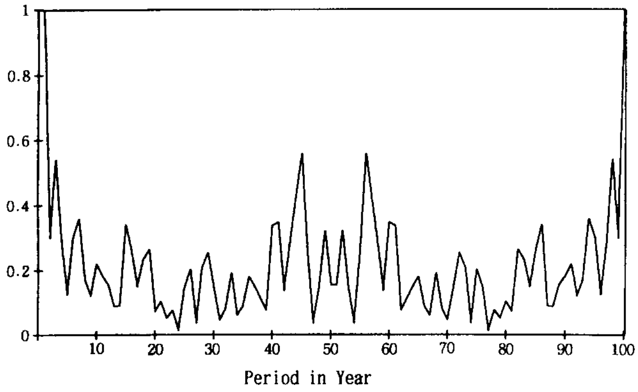

Figure 4 *FT* i.r. analysis of *Figure 3*

equal to those considering the coefficients up to four years previous (correlation coefficient = −0.96). As the result of this moving average process the curve between temperature and time (year) is smoothened. In other words, the curve becomes rather insensitive to whether the silk was produced in the year of death or not. *Figure 4* shows the estimated temperatures in the 12th century after consideration of the coefficients up to five years previous.

Comparing the fractions of glycine residues and the other two indices with the above estimated temperatures, we can find negative correlation between the fractions and the temperatures, and positive correlations between the other two indices and the temperatures. We can, therefore, conclude that warmer temperature produces silk with a lower fraction of glycine residues, a higher cross-section of filament, and a higher circularity coefficient.

Fourier transformation of *Figure 3* was done and the result is shown in *Figure 4*. As seen from the figure, the Fourier component corresponding to the period of 30 years is not so large as those of 3 and 44 years. The oscillations of the three quantities against year in *Table 2* are, therefore, reasonable.

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