SHORT COMMUNICATIONS

Proton Magnetic Resonance in Cocoon

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As the application of the nuclear magnetic resonance, we made a preliminary measurement of the proton resonance in cocoon in order to obtain information about the general features of the thermal motions of molecules. The line shape, the width, and the second moments of the absorption curves were investigated in usual way[1].

Apparatus and Sample

Most part of the apparatus of NMR measurements is same as described by Gutowsky et al.[2], and has been published elsewhere[2]. The magnetic field applied was about 4000 gauss. The intensity of the rf-field, H_1, was about 0.1 gauss. The samples were slightly saturated by this strength of H_1, but we could not reduce H_1 to the lower level. Measurements were made at room temperature as well as at lower temperatures which were obtained by using the Gutowsky's type[2] Dewar vessel. The temperature of the sample was measured by a copper-constantan thermocouple placed 5 mm. apart from the sample.

Several species of cocoon produced by the Tokyo University of Agriculture and Technology were used as the samples which were hereditally different from each other. Measurements of the temperature dependence of the NMR spectrum were made for the species, Shuka×Ginrei. Samples of four species including two yellow, one white, and one red were measured at the room temperature. They have the genes as shown in Table II, and each of them was divided into two parts of the outer and the inner layers.

Temperature Dependence of the Line Shape

Fig. 1 shows the results of the measurements for Shuka×Ginrei at different temperatures,

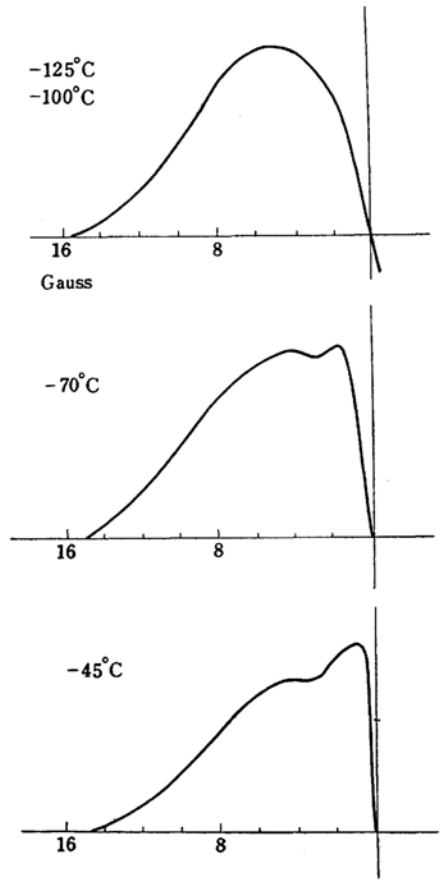

Fig. 1. Proton magnetic resonance in cocoon (species: Shuka×Ginrei). One half of the derivative of the absorption curve is presented as the function of temperature.

where a half of the derivative of each absorption curve is presented. It is noted in the figure that the observed spectrum shows a compound structure, and, moreover, that the relative intensity of the central peak with respect to that of the outer shoulder is much reduced at the lower temperatures, and not detectable at −100°C. The line widths of both the central- and the outer-peaks are not much changed by the temperature.

Besides the principal aminoacids of the silk fibroin, glycine (44%), alanine (26%), and tyrosine (13%), the native cocoon contains sericin. Hence, apart from the amido hydrogens, silk fibroin will contain approximately

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1) General aspect of the proton magnetic resonance in solid will be obtained in Chapter 6 of "Nuclear Magnetic Resonace" by E. R. Andrew, Cambridge University Press, Glasgow (1955), p. 151.

2) H. S. Gutowsky, L. H. Meyer and R. E. McClure, *Rev. Sci. Inst.*, **24**, 644 (1953).

3) S. Fujiwara and S. Hayashi, *Rep. of University of Electro-Communications*, No. 8, 96 (1956).

36% of the protons in methylene groups, but also about 27% in methyl groups, and 36% in CH, OH, or aromatic positions.

Although one may interpret that the existence of a compound structure (as in the cases of polyethylene and polytetrafluoroethylene[4,5] does not necessarily imply that there are two separate regions differing in their degree of motion as proved for natural rubber[6], polymethylmethacrylate[7] and so on, however, we assume that the structure of the observed spectra is due to the net contribution of the protons in different phases, very motional and rigid, since the relative intensity of the central peak is so strongly temperature dependent, and its width is almost independent.

Table I gives the characteristic figures of the spectrum.

As the component I is observable even at −70°C, it can not be attributed to the sorbed water which might be existent in the sample. The line width of II is invariant with temperature over a range of about 130°C, and if our assumption on the structure of the line

TABLE I. NMR LINE WIDTH OF COCOON (SHUKA×GINREI)

Component	Line width (in gauss)			
	<−100°C	−70°C	−45°C	21°C
I(narrow)	(−)	(0.8)	0.8	0.8
II(broad)	8.0	8.0	8.0	8.0

The value for I at −70°C was difficult to determine exactly, since its intensity was low.

TABLE II. CHEMICAL COMPOSITION AND THE NMR DATA FOR SEVERAL SPECIES OF COCOON AT 21°C

Sample		Layer	Color	Sericine	Amido-N	Carbon	Amido-N	Carbon	$\langle \Delta H^2 \rangle$	$(\Delta H)_{max.}$ sl. for component II in gauss	Weight % of component I.
				Sericine %	%	%	%	%	gauss²		
Osmt × Ci	C	outer	yellow	36.2	0.63	47	0.36	47	9.0	6.4	10.0
		inner	white	17.2	0.45	48	0.35	48	10.8	7.0	12.5
	Ci	outer	white	32.8	0.65	47	0.33	48	9.5	7.0	11.0
		inner	yellow	16.5	0.46	48	0.34	47	9.8	7.5	15.7
	+Ci	outer	white	30.2	0.68	47	0.34	48	9.7	7.0	16.0
		inner	white	15.4	0.48	49	0.36	47			
Pk	Pk+c	outer	red	30.0	0.64	47	0.35	48	9.7	7.9	12.0
		inner	white	17.7	0.51	48	0.33	48	11.6	8.3	14.5

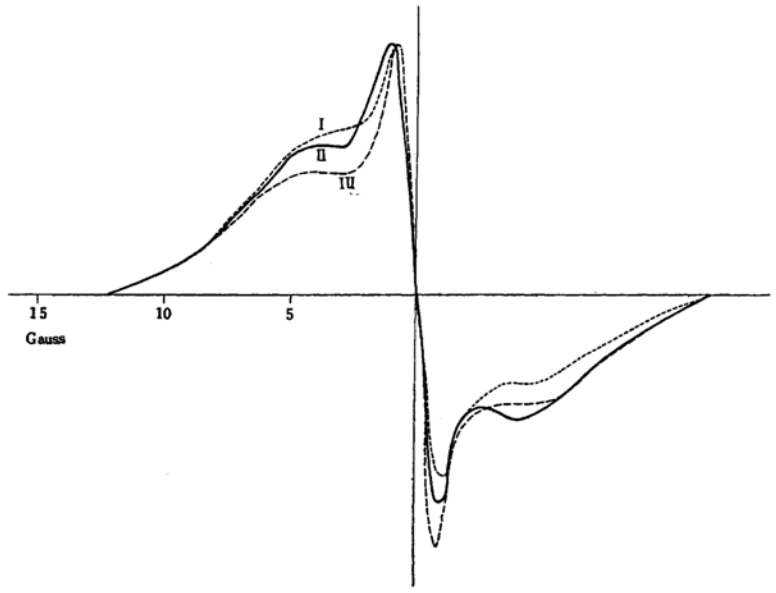

Fig. 2. Derivatives of the NMR absorption spectra for different species of samples at 20°C.
I Shuka×Ginrei, II Osmt, III Pk

4) C. W. Wilson and G. E. Pake, *J. Poly. Sci.*, **10**, 503 (1953).
5) C. W. Wilson and G. E. Pake, *J. Chem. Phys.*, **27**, 115 (1957).
6) H. S. Gutowsky and L. H. Meyer, ibid., **21**, 2122 (1953).
7) J. G. Powles, *Proc. Phys. Soc.*, **69B**, 281 (1956).

shape is correct, this will suggest that the protons in this component are effectively rigid.

The second moments at -100 and $-125°C$, where no central peak was observale, were 26.8 and 26.1 gauss^2, respectively, and those at $-75°$, $-45°$ and $+21°C$ were 26.1, 24.3 and around 10 gauss^2, respectively, which could be explained by the variation of the relative intensities of I and II.

Line Shapes for Different Species of Cocoon

Measurements were made for the samples different in species. The results of the measurements were shown in Table II, where the results of the chemical analyses were also shown. Though there is not much difference either in the chemical composition or in the features of the NMR data, among the samples of different species, we notice that the behavior of saturation is different: Fig. 2 shows the normalized derivative curves for the samples, where no saturation effect is observed in the record of Pk, since the each half of the spectrum is completely symmetric with respect to the center of the base line, whereas, in Shuka×Ginrei, the intensity of the right-hand side half is strongly reduced.

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