Molecular Motion in Poly(amino acid). III. Proton Broad Line NMR of Amorphous Silk Fibroin

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In preceding papers,^{1,2} it was clarified that the force of intermolecular hydrogen bonding in the β -form crystal of silk fiber decreased by heating above 140°C and that the distances between the pleated sheets then increased with molecular motion, while the silk fibroin molecules oriented parallel to the fiber axis in the amorphous part of the silk fiber.

In this paper, the molecular motion of the unoriented amorphous silk fibroin was investigated by the proton broad line NMR (B. L. NMR). B. L. NMR spectra were taken with a Varian WL-210 RF unit at a frequency of 30 MHz over the temperature range from -150°C to 200°C. Preparation of the amorphous silk fibroin film of *Bombyx mori* has been described elsewhere.¹ For measurements, the film prepared was packed randomly into a glass tube, dried in a vacuum of 10^{-5} mmHg for 2 h, and then sealed.

Figure 1 shows the first derivatives of B. L. NMR absorptions ($S'(x)_{\text{obs}}$) at various temperatures. In the figure, the intensities of spectra from 150°C to 90°C are two times those from 100°C to 200°C. Each curve shows one component from -150°C to -50°C. Above -30°C, the derivatives, however, were composed of two components. These derivatives decomposed, assuming that a narrow and a broad component were the first derivatives of Lorentz curve (eq 1) and of Gauss curve (eq 2), respectively.³

$$F'(x) = A \cdot x / (B^2 + x^2) \quad (1)$$

$$G'(x) = C \cdot x \cdot \exp(-x^2/2D^2) \quad (2)$$

where A , B , C , and D are parameters. The para-

meters were selected to satisfy eq 3 by computer (HITAC 8700/8800, TODAI CENTRE).

$$| \{ S'(x)_{\text{obs}} - S'(x)_{\text{calc}} \} / S'(x)_{\text{obs}} | < 0.1 \quad (3)$$

where $S'(x)_{\text{calc}} = F'(x) + G'(x)$. In Figure 1 the dashed lines show the calculated curves of $F'(x)$ and $G'(x)$. $S'(x)_{\text{calc}}$, however, was not shown in Figure 1, because $S'(x)_{\text{calc}}$ and $S'(x)_{\text{obs}}$ almost overlapped with each other.

Figure 2 shows the plots of the maximum slope width ($\delta H_{m.s1}$) vs. temperature for the narrow and the broad components. The $\delta H_{m.s1}$ of the broad component was determined from the calculated curve ($G'(x)$) above 100°C, where $\delta H_{m.s1} = 2D$ from eq 2.

The broad component narrows in two transition steps. In preceding studies,^{4,5} it was proved that the narrowing of the broad component was due to rotations of the relatively long length of side chains in poly(amino acid)s, such as poly(γ -methyl L-glutamate), poly(γ -benzyl L-glutamate), poly(β -methyl L-aspartate) and poly(L-methionine) from about -40°C to room temperature, while narrowing due to the rotation of the short length of side chain such as alanine residue occurs even below liquid N₂ temperature.⁶ Glycine, alanine, serine and tyrosine are the components of amino acid in the silk fibroin.⁷ From these facts, the narrowing at lower temperatures (-20~20°C) is due to the rotation of the relatively long length of side chains, *i. e.*, serine and tyrosine residues. On the other hand, the glass transition temperature measured by dilatometry was 175°C,¹ and the dispersion showing the $\tan \delta$ maximum at 175°C

Figure 1. Halves of the first derivative of B.L.NMR spectra for the amorphous silk fibroin sample.

Figure 2. Plots of line width vs. temperature for the amorphous silk fibroin sample: O, broad component; x, narrow component.

occurred gradually above 100°C in the temperature dependence of viscoelasticity.⁸ Thus, the narrowing at higher temperatures is due to the vibration of the main chain and the successive segmental motion of the main chain. According to Ma-

goshi,⁹ the amorphous silk fibroin molecules crystallize into β -form crystal above 180°C. Thus, the increase in $\delta H_{m.s.l}$ from 180°C to 200°C means the crystallization.

Hence the rotation of side chains caused a narrowing of the broad component rather than the appearance of a narrow component; and as mentioned above, the narrow component observed here is not due to the side chains. If the water was adsorbed in the sample, a sharp narrow component should be 40 mm gauss at above 0°C, with a width independent of the measured temperature.¹⁰ Conversely, the width of the narrow component was as much as 4 gauss at -30°C for the sample used, and narrows gradually to 1 gauss with increasing temperature. Thus, this narrow component is not due to the adsorbed water. In a physical investigation, polyurethane,¹¹ polyamide¹² and some synthetic poly(amino acids)^{13,14} showed a new dispersion (β_2) due to the

Figure 3. Infrared spectrum for the amorphous silk fibroin sample.

existence of tightly bound water. E. Baer, *et al.*,^{13,14} concluded that the tightly bound water was not easily removed by vacuum drying and forms bridges between pairs of amide and peptide groups, namely forms polymer—water complex, and that the tightly bound water has no properties of normal water. There is no normal water in the used sample as described above, but the tightly bound water will be contained, since its removal is very difficult. It is possible to consider that the narrow component is attributable to the motion of the polymer—water complex. Furthermore, there is a considerable number of NH groups free from intermolecular or intramolecular hydrogen bonds formed in the process of the sample preparation, whose infrared absorption

band appeared at 3480 cm^{-1} (Figure 3). This means that several sequential residues free from hydrogen bond exist in the silk fibroin molecules. Thus, it is also reasonable to consider that the narrowing is due to the motion of these sequential residues.

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