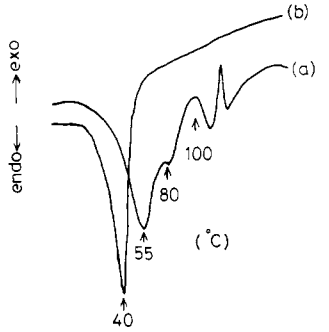

Figure 1. Observed DTA curves for (a) **3a-1** and (b) **3a-2** (20 °C/min).

Scheme I

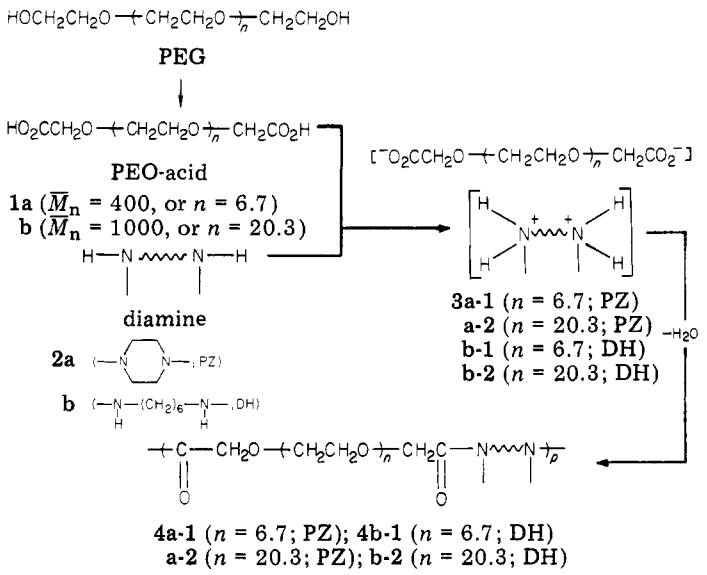

Table I
Thermal Polymerization[a] of the Salts of PEO-acid

salt	product polymer	η_{sp}/C[b]	N anal. calcd	N anal. found
3a-1	**4a-1** (white wax)	0.141[c]	5.85	5.66
3a-2	**4a-2** (white solids, mp 32 °C)	0.259	2.59	2.18
3b-1	**4b-1** (pale yellow wax)	0.124	5.85	6.16
3b-2	**4b-2** (white solids, mp 29 °C)	0.310[d]	2.63	2.64

[a] At 200 °C for 6 h. The yields were quantitative. [b] At a concentration of 5.0 g/L in H_2O (30 °C). [c] A GPC analysis of this polymer indicated $\bar{M}_n$ = 13 000 with $\bar{M}_w/\bar{M}_n$ = 1.55. [d] GPC analysis: $\bar{M}_n$ = 20 000; $\bar{M}_w/\bar{M}_n$ = 1.76.

break = 150%, and Young's modulus = 7 kg/mm^2. We are now studying copolycondensations using other amine salts of PEO-acid.

Acknowledgment. We are indebted to both Nippon Shokubai Kagaku Kogyo Co. Ltd. (Osaka, Japan) and Kawaken Fein Chemicals Corp. (Tokyo, Japan) for the sample of PEO-acid.

References and Notes

(1) Some preparations of carboxyl-terminated poly(oxyethylene) have been reported. However, the yield of carboxyl groups was not quantitative. For example: Geckeler, K.; Bayer, E. *Polym. Bull.* **1979**, *1*, 691.
(2) Saito, H.; Nozue, M.; Takizawa, H. Japan Kokai S53-141219, 1978.
(3) M_n range = 380–420; η_{sp}/C = 0.030 (5.0 g/L in H_2O at 30 °C).
(4) M_n range = 950–1050; η_{sp}/C = 0.053 (5.0 g/L in H_2O at 30 °C).
(5) Moore, J. A. "Macromolecular Syntheses"; Wiley: New York, 1977; Collect. Vol. 1, p 317.
(6) The spectroscopic data were very similar to those of **3a-1**.
(7) After recrystallization, generally, the PEO components of the recrystallized parts may become narrower in molecular weight distribution than the parent one. Thus, the uncrystallized parts involve ones with rather wider molecular weight range, which may cause the difficulty in recrystallization.
(8) Spectroscopic and analytical data, being similar to those of **3a-1**, were obtained to support the structures of both **3b-1** and **3b-2**.

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Conformational Characterization of Silk Fibroin in Intact *Bombyx mori* and *Philosamia cynthia ricini* Silkworms by ^{13}C NMR Spectroscopy

We report here that the intact ^{13}C NMR approach has been successfully applied to the conformational characterization of silk protein, mainly silk fibroin, stored in the middle silk gland of silkworms before spinning. It has been confirmed that the conformation of silk fibroin in the solid state from *B. mori* depends strongly on the conditions of sample preparations: silk fibroin mildly dried at room temperature becomes the silk I type (loose helix); the silk II type (antiparallel β-sheet structure) appears as a more stable form in samples dried at higher temperature or under mechanical stress.[1] The intact NMR approach gives unique and novel information about the structure of the native protein in solution because the samples are free from any external forces. Here, the conformation of liquid silk stored in silk glands is characterized by a ^{13}C NMR spectroscopic comparison of intact *B. mori* with *P. c. ricini*,[2] whose amino acid composition is appreciably different.

Experimental Section. The ^{13}C NMR spectra of intact *B. mori* and *P. c. ricini* silkworms were observed with a JEOL FX-200 NMR spectrometer operating at 50 MHz.[3,4] The spectral width was 12 000 Hz, and 8K data points were used. It was not necessary to employ sample spinning or magnetic field locking because of the high stability of the superconductive magnet. The ^1H nuclei were decoupled only during the sampling time of 0.34 s to avoid an increase of temperature in the sample tube. In the ^{13}C NMR measurements of the liquid silk in urea solution, complete ^1H decoupling was used. Chemical shifts are reported in ppm downfield from external Me_4Si.

Results and Discussion. Figure 1 shows the ^{13}C NMR spectra of the silk gland portion of the intact mature larvae of *B. mori* and *P. c. ricini* and of the abdomen of a *P. c. ricini* pupa. The peak assignments have been reported previously;[3] mobile components such as the silk protein, triglyceride, and α,β-trehalose in the silkworms give well-resolved spectra. It has been confirmed that the amino acid peaks are essentially due to the silk protein. This conclusion is based on measurements of the ^{13}C NMR spin–lattice relaxation time, T_1, using 180–τ–90° pulse sequences in intact silkworms. The T_1 values of the amino acid carbons in intact *B. mori* are considerably shorter than those of the corresponding free amino acids. For example, the NT_1 values of the α-carbons of the Gly, Ala, and Ser residues in the spectrum, where N is the number of hydrogen atoms directly attached to specified carbon atoms, are ca. 0.20 s and they are near the corresponding values

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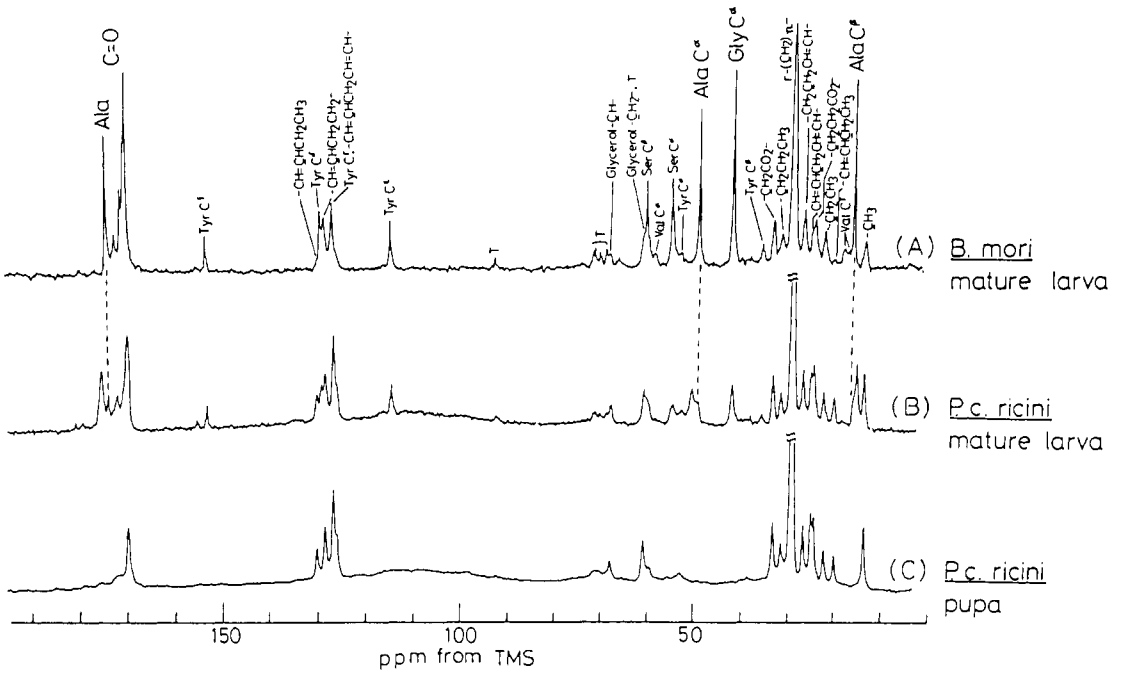

Figure 1. ^{13}C NMR spectra of the silk gland portion of both intact *B. mori* (A) and *P. c. ricini* (B) mature larvae and of the abdomen of *P. c. ricini* pupa (C). The number of accumulations was 4000–10 000. Experimental details are given in the text.

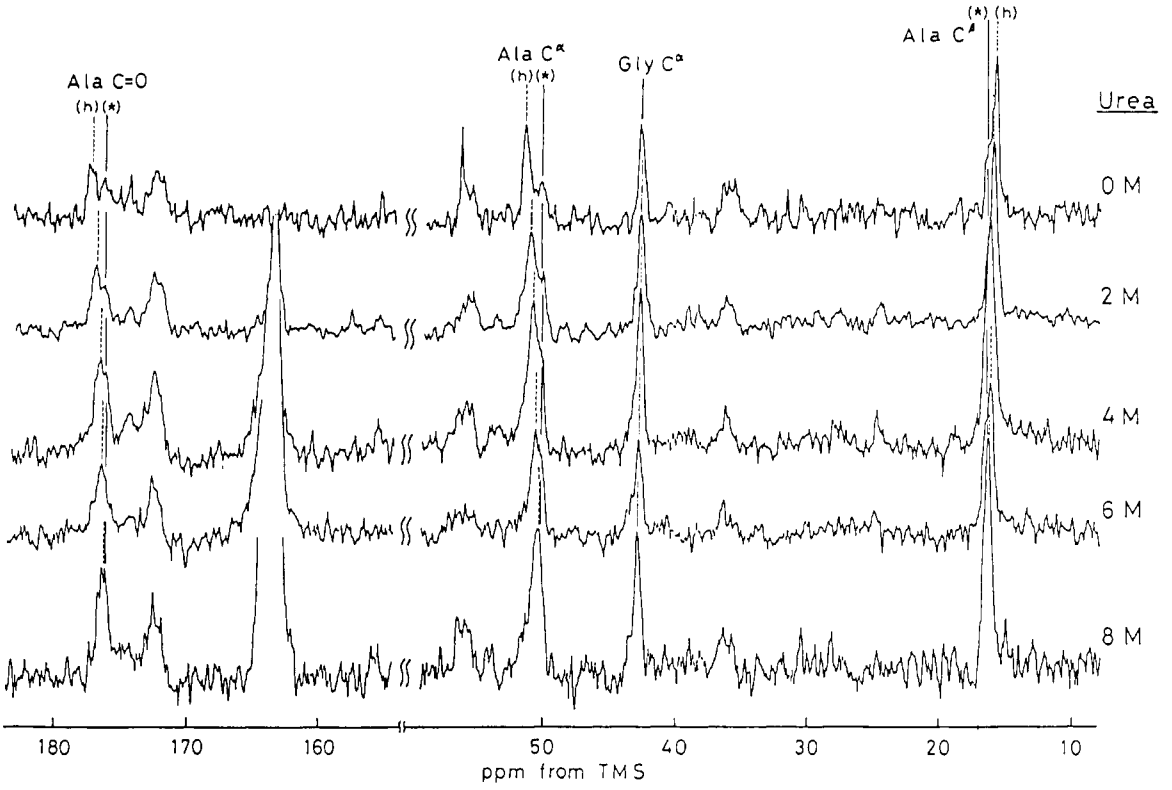

Figure 2. ^{13}C NMR spectral changes of the liquid silk extracted from the mature larva of *P. c. ricini* as a function of urea concentration. The peaks marked h are attributable to the α-helical peak and the peaks marked with asterisks are attributable to the random coil or silk I type peak as described in the text. The number of accumulations was 1000–2000.

of *B. mori* silk fibroin solution obtained from the cocoon.[4] It should be emphasized that the shapes of the peaks from the Ala residue from *P. c. ricini* silkworms are doublets or asymmetric, while the corresponding Ala peaks of *B. mori* are all singlets, although the C^{α} peaks from the Gly residues in both silkworms are singlets. Moreover, the peak positions of the low-field component of Ala C^{β} and of the high-field components of Ala C^{α} and C=O coincide with those of *B. mori*. Since the spectrum of the pupa gives no peaks in the corresponding shielding region, the doublet peaks must come from the Ala residue.

In order to assign these peaks, urea was added as a denaturing reagent to the liquid silk extracted from the mature larva of *P. c. ricini* as shown in Figure 2. With increasing concentration of urea, the high-field component of the Ala C^{β} peak marked by h shifts to lower field, and the low-field peaks h of Ala C^{α} and C=O shift to higher fields. Other peaks marked by asterisks and the Gly C^{α} peak do not shift. As a result, the peaks of the Ala residues coalesce in 8 M urea solution. When urea was added to

the liquid silk of *B. mori*, no spectral change was observed. Since the presence of the α-helix in *P. c. ricini* silk fibroin has been confirmed from ORD measurements by Iizuka,[5] the low-field components in both the C^{α} and C=O peaks of the Ala residue and the high-field component in the Ala C^{β} peak are assigned to the α-helical portion of the chain. Although unambiguous assignment of other components marked with asterisks is not possible from only these data, they might be assigned to the random coil or silk I type portion rather than the silk II type portion since no peaks occur in the usual high-resolution NMR spectrum of silk II type fibroin.[6] These assignments coincide with a theoretical prediction of the conformation-dependent ^{13}C NMR chemical shift of polypeptides by Tonelli.[7] From the peak ratios of both the C^{α} and C^{β} peaks, the fraction of Ala residues which takes part in the α-helix formation is determined to be 0.65 for *P. c. ricini* mature larva, although the fraction is slightly larger (0.7) when the C=O doublet peak is used for the estimation. Moreover, we can conclude that there is no α-helical portion in the silk fibroin stored in the middle silk gland of *B. mori*.

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