Film Formation and Structural Characterization of Silk of the Hornet *Vespa simillima xanthoptera* Cameron

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We extracted silk produced by the larva of the hornet *Vespa simillima xanthoptera* Cameron from its nest. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the extracted hornet silk showed four major components with molecular weights between 35 and 60 kDa. The main amino acid components of the hornet silk protein were Ala (33.5%), Ser (16.9%), Asp (8.5%) and Glu (8.1%). The hornet silk could be dissolved in hexafluoroisopropyl alcohol (HFIP) at 25 °C without incurring molecular degradation. A transparent film of hornet silk was obtained readily by the formation of a cast upon drying of the hornet silk in the HFIP solution. Residual HFIP solvent was removed from the film by extraction with pure water. Solid-state ^{13}C NMR and FT-IR measurements revealed that the secondary structures of hornet silk proteins in the native state consisted of coexisting α-helix and β-sheet conformations. The β-sheet to α-helix ratio, which was changed by processing, was mainly responsible for the silk's thermostability.

Key words: Wasp Cocoon Protein, Amino Acid Composition, Molecular Conformation

Introduction

Our group has been investigating the properties and molecular structures of various products secreted by insects, such as waxes, chitins, and silks (Kameda, 2004a, b; Kameda *et al.*, 2005, 1999a, b; Inoue *et al.*, 2005, 2004). Although silk has been widely studied in the Lepidoptera (silkworms), because by far the largest quantity comes from the silkworm *Bombyx mori*, special attention has been given to the silk produced by the Hymenoptera (ants, bees and hornets) (Kameda, 2004b; Flower and Kenchington, 1967; Gomes *et al.*, 2004; Ishay and Kirshboim, 2000; Ishay *et al.*, 2003; Joseph and Ishay, 2004; Yamada *et al.*, 2002, 2004; Silva-Zacarin *et al.*, 2003; Rudall and Kenchington, 1971). Previous characterizations of silk from the Hymenoptera have indicated that its amino acid composition differs from that produced by silkworms (Yamada *et al.*, 2002, 2004; Espelie and Himmelsbach, 1990). Moreover, the silk secreted by hymenopteran larvae is considered to exhibit a conformation that is different from those of the fibrous structures produced by both spider and insect adults and by other insect larvae (Silva-Zacarin *et al.*, 2003). Therefore, we expected that silks from the Hymenoptera would have intrinsic properties different from those of the silks produced by the Lepidoptera. In the light of this expectation, we wanted to further explore the usefulness of silk from the Hymenoptera as a new natural material (Kameda, 2004b).

Ishay and coworkers measured the spontaneous electric current flow in the silk of pupae of the Oriental hornet (hornet silk) as a function of temperature (Ishay *et al.*, 2003; Kirshboim and Ishay, 2000). They detected a clear correlation between temperature rise and the increase in electric current forming in the hornet silk. Although some other unique characteristics of hornet silk in the native state were reported by Ishay and his coworkers (Joseph and Ishay, 2004), there have been no efforts to produce useable materials, such as biofilms, from this potentially useful resource. Moreover, no detailed structural studies of hornet silk at the atomic level have been carried out.

Some kinds of hornet build large nests. *Vespa simillima xanthoptera* Cameron is one of the most common hornets in Japan. This hornet is a social

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insect that builds a new nest each summer. The largest nests are over 1 m in diameter. The hornet's mature larval stage (5th instar) goes into the pupal stage after enwrapping itself in a silk weave (hornet silk) comprised of protein fibers. Inside each large nest a very large volume of hornet silk caps (cocoon) can be seen. This gives the silk of *V. simillima xanthoptera* Cameron a quantitative edge in terms of its potential availability as a material. However, in no study it was ever tried to produce useable materials from the silk of *V. simillima xanthoptera* Cameron. To produce useable materials from hornet silk we need a detailed understanding of the processing conditions. Moreover, although knowledge of the amino acid composition, molecular conformation, and properties of the silk of hornet *V. simillima xanthoptera* Cameron and their manipulation may be necessary to prepare materials of desired functionalities, little attention has been given to this point.

In this study, to establish a process for forming a film of the silk of the hornet *V. simillima xanthoptera* Cameron as a new natural material, we examined the dissolving ability of this silk. We also investigated the factors affecting the molecular mass of hornet silk in the dissolving process, and we describe a procedure for obtaining biofilm from hornet silk without oncurring molecular degradation. Furthermore, we clarified the structure and properties of the film that was generated.

Experimental

Hornet cocoons

The nests of *V. simillima xanthoptera* that we used were collected in Iwate Prefecture, Japan, in August and September 2004. The larvae, pupae, and adults were removed from the nests, which were then dried. The cocoons were then collected from the dried nests. Although we intended to collect only the cocoons, we also collected some chewed wood fibers that were used to build the cell walls of the nest and were adhered to the cocoons. To remove the wood fibers and obtain the purified hornet silk, the cocoons were dissolved in aqueous 7.2 M lithium bromide (LiBr, Wako Pure Chemical Industries, Ltd.) for 15 min at 25 °C, and then filtered. The filtrate was dialyzed in a cellulose tube for 4 d to remove the salt; the outer distilled water was refreshed every day. Finally, the aqueous protein solution was freeze-dried.

Solubility of hornet silk in solvents

First, an adequate amount of hornet silk was dissolved in arbitrary quantities of 9.0 M aqueous LiBr solution by shaking at 100 rpm at 25 °C. After the silk completely dissolved, the UV absorbance at 280 nm was read with a GeneQuant Pro UV spectrometer (Amersham Pharmacia Biotech) in 10-mm 70-μL plastic cells. On the basis of this preliminary measurement, we calculated the accurate weight of hornet silk corresponding to an absorbance of 1.0; we found that the UV absorbance was 1.0 when 100 mg of hornet silk was dissolved in 20 mL of the LiBr solution. We then dispersed 100 mg of hornet cocoon in the native state in 20 mL aqueous 5.2, 7.2 or 9.0 M LiBr solution over various periods of time and observed the absorbance of the silk solution at 280 nm in order to compare dissolving abilities at various LiBr concentrations over various times. A relative absorbance of 1.0 indicated 100% dissolution.

100 mg of hornet cocoons in the native state were dispersed in 4 mL of trifluoroacetic acid (TFA), dichloroacetic acid (DCA), or hexafluoroisopropyl alcohol (HFIP) by shaking at 100 rpm at 25 °C. The solubility was checked frequently by visual observation.

Formation of hornet silk film

Both native-state and purified hornet silk were soluble in TFA and HFIP. 100 mg of purified hornet silk were dissolved in 4 mL of TFA for 5 min at 0 °C, or in HFIP for 1 d at 25 °C. After complete dissolution, a film of hornet silk was prepared by spreading the TFA or HFIP solution of hornet silk over a polystyrene plate and leaving it to dry for 1 d at room temperature. Finally, the films were rinsed in water to remove the residual solvents.

Amino acid analysis

The hornet silk sample was hydrolyzed in 6 M HCl at 110 °C for 22 h. The amino acid composition of the hydrolyzed sample was determined by amino acid analysis (Hitachi Automatic Amino Acid Analyzer Model L-8500). Amino acid calibration mixture (Ajinomoto Takara Corp. Inc., Tokyo) was used to prepare the standard solution for amino acid analysis.

Electrophoresis

Protein samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with an electrophoresis unit (Atto Corporation, Tokyo, Japan). A 15% polyacrylamide gel (NPU-15L, Atto Corp.) was used. The molecular mass standard used was a BioRad Kaleidoscope prestained standard (cat. # 161-0375). Sample 1 for SDS-PAGE was prepared as follows: 100 mg of native cocoon were dissolved in 20 mL of 7.2 M aqueous LiBr solution at 25 °C within 15 min, and diluted with 4 times the volume of distilled water. Sample 2 was prepared as follows: 20 mL of 9.0 M aqueous LiBr solution with 100 mg of native cocoon were shaken at 60 °C for 3 h and then diluted with 4 times the volume of distilled water. Sample 3 was prepared as follows: 2 mL of HFIP solvent with 100 mg of native cocoon were shaken at 25 °C for 3 d and then the solvent was evaporated. After removal of the solvent, the hornet silk was dissolved in 7.2 M aqueous LiBr solution at 25 °C within 15 min, and diluted with 4 times the volume of distilled water. These three samples were stable in diluted solution and no precipitates were recognized. Each sample was mixed with an equal volume of sample buffer [Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl), 0.125 M; 2-mercaptoethanol (2-Me), 10%; SDS, 4%; sucrose, 10%; bromophenol blue, 0.004%]. After the solutions had been boiled at 95 °C for 3 min, SDS-PAGE analysis was carried out. 10 μl of each of the sample solutions were loaded for SDS-PAGE.

Solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR)

Solid-state ^{13}C NMR spectra were obtained on a CMX 300 spectrometer (Chemagnetics, Fort Collins, CO, USA) at the Forestry and Forest Products Research Institute, Tsukuba, Japan, operating at a ^{13}C NMR frequency of 75.4 MHz. The samples were spun at the magic angle at 5.0 kHz in a solid-state probe in a 7.5-mm zirconia rotor for the CMX 300. All spectra were obtained by using ^{1}H NMR 90° pulse lengths of 5.0 μs, with a cross-polarization time of 2.0 ms and 60-kHz CW proton decoupling. A recycling time of 3 s was used. All spectra were calibrated by using adamantane as a standard (the CH_2 peak at 29.5 ppm gives shift values referenced to TMS carbon at 0 ppm).

Fourier-transform infrared spectroscopy (FT-IR)

All infrared absorption spectra were recorded with a Jasco Herschel-350 Fourier transform infrared spectrophotometer. All measurements were recorded at a resolution of 4 cm^{-1}, and scans were made 32 times.

Differential scanning calorimetry (DSC)

A TA Instruments Q100 differential scanning calorimeter utilizing N_2 gas was used to assess the melting and thermal denaturation of the hornet silk for each processing condition. The scanning temperature was raised from 0 to 390 °C at a rate of 10 °C/min.

Results and Discussion

Solubility of hornet silk in aqueous LiBr solution

An aqueous LiBr solution had already been proposed as a useful solvent for silkworm silk fibroin (Asakura and Yao, 2002; Yang *et al.*, 2004; Zhao *et al.*, 2003). Thus, the dissolution of hornet silk of *V. simillima xanthoptera* in the native state was carried out in aqueous LiBr solution under various dissolving conditions. Fig. 1 shows the degree of dissolution of hornet silk in various concentrations of aqueous LiBr solution as a function of shaking time. The dissolving temperature was 25 °C.

We found that the dissolving ability was significantly dependent on both concentration of LiBr and time. With LiBr concentrations of 7.2 and 9.0 M, the protein concentration in the solution dramatically increased with increasing shaking time and asymptotically reached approx. 100%

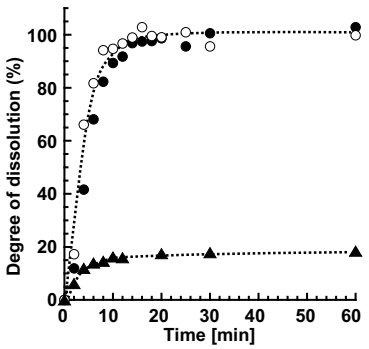

Fig. 1. Plots of degree of dissolution (%) in 5.2 M (▲), 7.2 M (○) and 9.0 M (●) LiBr aqueous solution of hornet silk at 25 °C as a function of shaking time.

within 15 min. In contrast, the change in the protein concentration at a LiBr concentration of 5.2 M was slight: after shaking for 12 h, only approx. 20% of the total amount of cocoon was dissolved. This result demonstrated that a LiBr concentration of at least 7.2 M was needed for the hornet silk to completely dissolve within a practicable time.

From SDS-PAGE analysis, the protein components dissolved in 5.2 M LiBr solution were found to be the same as those dissolved in 7.2 or 9.0 M LiBr solution. The results of SDS-PAGE analysis will be presented later.

Purification of hornet silk

Development of a suitable method for separation, isolation, and purification of the hornet silk from the nest is important for producing a useable material. Inside the nest of the hornet, the cocoons adhere to the plant fibers, because the paper-like cell walls in the nest are made of chewed wood fibers (Espelie and Himmelsbach, 1990). As described above, in its native state the cocoon protein dissolved in aqueous LiBr solution. In contrast, the wood fiber was insoluble in this solution. We used this difference in solubility to extract the hornet silk from the nest. Cocoons in the native state (*i.e.* mixed with wood fibers) were dissolved in 7.2 M LiBr solution. After the solution had been shaken for 15 min at 25 °C, the insoluble residue of wood fibers was removed by filtration. The filtrate was dialyzed in distilled water. Finally, the aqueous protein solution was freeze-dried, and a purified powder of hornet silk was obtained.

Film formation from hornet silk

We found that the hornet silk could be dissolved at room temperature in some halogenated organic solvents, such as trifluoroacetic acid (TFA) within a few minutes, dichloroacetic acid (DCA) within a few hours, and hexafluoroisopropyl alcohol (HFIP) within a few days. TFA and DCA had already been proposed as useful solvents for polypeptides (Kameda *et al.*, 1996; Yamashita *et al.*, 1979), and HFIP for the regeneration of *B. mori* silk fibroin and spider silk (Lock, 1993; Asakura and Yao, 2002; Kim *et al.*, 2005). We were the first to discover that evaporation of the HFIP or TFA solutions yield a flexible transparent film from the hornet silk. The transparency of the film varied with the type of halogenated organic solvent used, probably because of differences in volatility. The transparency of the film prepared in HFIP solution was best. On the other hand, no film was formed from DCA solution, owing to the poor volatile solvent characteristics of DCA.

Because HFIP solvent is harmful, the residual HFIP solvent in the film must be removed from the film to be utilized as a material. The film prepared from HFIP solution was dried in vacuum at room temperature for 1 week, and its FT-IR spectrum was then observed (Fig. 2A). Comparison with the FT-IR spectrum of HFIP solvent (Fig. 2C) revealed absorption bands attributable to the HFIP solvent (Fig. 2A), indicating that the HFIP solvent remained in the film after drying. We found that the residual HFIP in the film was readily and completely removed by rinsing in water. The bands attributable to the HFIP solvent disappeared from the FT-IR spectrum of the film after rinsing in water at room temperature for 1 d (Fig. 2B).

Moreover, comparison between Figs. 2A and 2B revealed that the line shapes of the FT-IR spectra were different before and after rinsing in water. It is known that the absorption bands obtained from FT-IR spectra are sensitive to the molecular conformation (Chen *et al.*, 2001). Further discussion

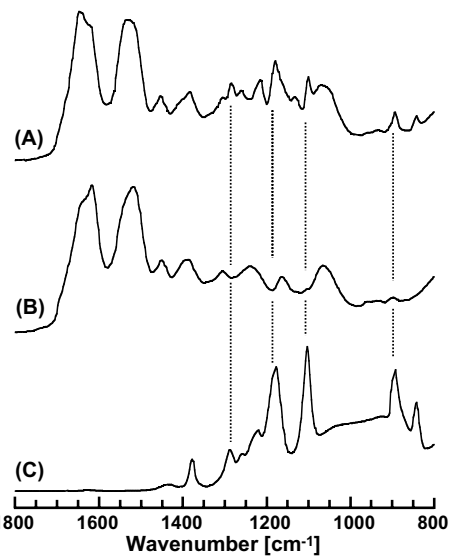

Fig. 2. FT-IR spectra of silk film of the hornet *Vespa simillima xanthoptera* Cameron, prepared from HFIP solution; (A) film dried in vacuum at room temperature for 1 week; (B) film rinsed in water at room temperature for 1 d. (C) FT-IR spectrum of HFIP solvent shown for comparison.

about molecular conformation will be presented later.

SDS-PAGE analysis of hornet silk

To confirm the validity of using aqueous LiBr solution and HFIP for processing hornet silk and to gain a more detailed understanding of the processing conditions, we used SDS-PAGE analysis to examine the effect of these solutions on the molecular chains of hornet silk during the dissolving process. The results of SDS-PAGE analysis for samples 1 and 2 are shown in Fig. 3. The proteins migrated mainly as two bands, with molecular weights of approx. 35 and 60 kDa, and each band was represented by double bonds (doublet). Therefore, a total of four main protein components were present. These four protein bands were marked as bands 1, 2, 3, and 4.

A previous study by Yamada *et al.* (2004) showed that SDS-PAGE of the cocoon fiber of the parasitic wasp *Apanteles glomerata* after removal of the glue protein gave a single band with a molecular mass of about 500 kDa. This is very different from our result, even though their cocoons were also produced by hymenopteran larvae. Therefore, in our study it was probable that the native molecule was degraded to small molecules during dissolution in the aqueous LiBr solution. However, no such degradation occurred, as was shown by the SDS-PAGE results. In SDS-PAGE (Fig. 3), the lane representing sample 2 (lane 2) appeared different from that for sample 1 (lane 1), showing a weaker intensity of band 1. This result indicated that mixing for a longer time at a higher temperature accelerated the degradation of the hornet silk. Here, it must be noted that the degradation of hornet silk by aqueous LiBr solution was only partial and was generated only from the protein attributed to band 1. The fact that the intensity of band 1 in lane 1 was strong indicates that shaking for a short time (up to 15 min) at a lower temperature (less than 25 °C) prevented degradation. Therefore, we can confirm that the four major bands – 1, 2, 3, and 4 – were not caused by small degradation products.

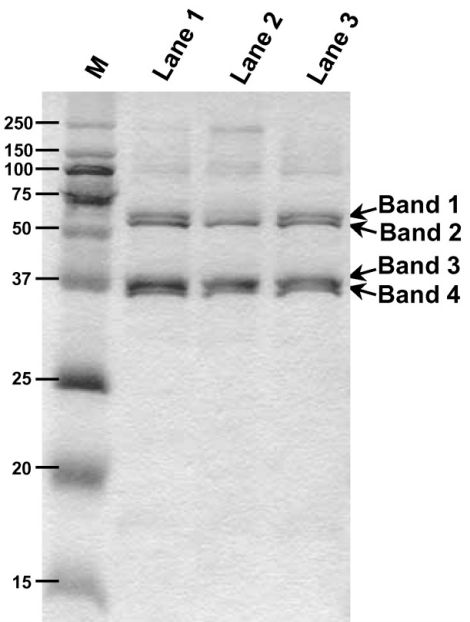

Fig. 3. SDS-PAGE of cocoon protein from *Vespa simillima xanthoptera* Cameron. Lanes 1, 2, and 3 correspond to hornet silks dissolved in 7.2 M LiBr aqueous solution for 15 min at 25 °C (sample 1), 9.0 M for 3 h at 60 °C (sample 2), and HFIP for 3 d at 25 °C, respectively. Lane M is the molecular weight marker. Arrows at right indicate the four major hornet silk proteins.

Moreover, the above results clearly indicated that dissolving hornet silk in aqueous LiBr solution for shorter times and at lower temperatures is indispensable for purification without oncurring molecular degradation. The best procedure is to dissolve the silk in 7.2 M LiBr solution and shake for up to 15 min at a temperature lower than 25 °C.

Fig. 3 (lane 3) shows the results of SDS-PAGE of a solution from a HFIP cast film (sample 3); it is not noticeably different from the results for sample 1. This indicates that no degradation occurred during shaking in HFIP solution for 3 d at room temperature; HFIP is therefore a useful solvent for the processing of hornet silk.

From these results, we can confirm the validity of using aqueous LiBr solution and HFIP for the processing of hornet silk.

Analysis of amino acids in hornet silk

The amino acid composition of the hornet silk from *V. simillima xanthoptera* is presented in Table I, together with data on the cocoon protein of another wasp, *Polistes annularis* (Espelie and Himmelsbach, 1990). The amino acid compositions of the hornet silk of both wasps were similar, indicating that the order Hymenoptera is phylogenetically conserved. Both had few Gly units and high contents of Ala, Ser, and acidic residues. Ala and

Table I. Amino acid compositions (%) of the cocoon proteins of *Vespa simillima xanthoptera* Cameron (this work) and *Polistes annularis* (Espelie and Himmelsbach, 1990).

	V. simillima xanthoptera Cameron	Polistes annularis
Ala	33.5	33.6
Ser	16.9	19.6
Asp	8.5	6.0
Glu	8.1	6.5
Leu	5.3	4.5
Lys	5.6	3.1
Arg	4.7	8.1
Gly	4.5	7.5
Thr	4.2	3.1
Cys	3.3	–*
Ile	1.9	1.6
Val	1.9	4.6
Pro	1.4	0.7
Met	0.2	0.3
Tyr	–	0.3
Phe	–	0.3
His	–	0.1

* Not detected.

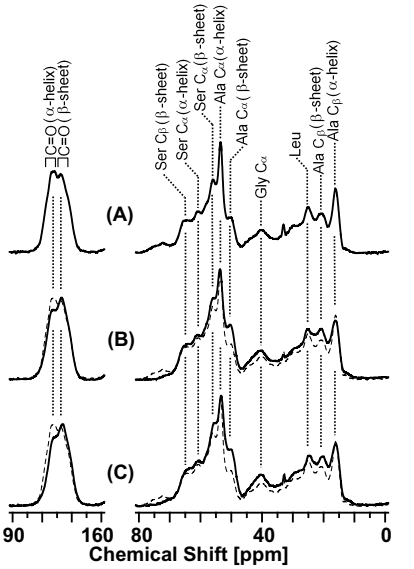

Fig. 4. ^{13}C CP/MAS NMR spectra of silk of the hornet *Vespa simillima xanthoptera* Cameron. Dashed lines show the spectra of the native state; solid lines show those of the native state (A), purified powder (B), and film (C). The peak intensities for each spectrum were normalized by the intensity of the peak of Ala C_α (α-helix).

Ser comprised more than half the residues found in the hornet silk.

Solid-state ^{13}C NMR experiment

From SDS-PAGE, we found that the hornet silk consisted of a protein mixture. Often solid-state NMR, FT-IR, and DSC measurements are not useful in the assessment of such protein mixtures because of the complicated nature of the observed spectra. However, because materials are used in the mixed-protein state, we consider that these measurements will still prove useful. Previously, Espelie and Himmelsbach (1990) demonstrated the ^{13}C CP/MAS (cross polarization/magic angle spinning) spectrum of hornet silk. However, no detailed structural analysis was carried out through examination of ^{13}C chemical shifts. Consequently, little progress has been made in efforts to generate an understanding of the relationship between the structure of hornet silk and its corresponding NMR chemical shifts.

Figs. 4A to C show the ^{13}C CP/MAS NMR spectra of the silk of *V. simillima xanthoptera* in the native state, purified powder and HFIP film after rinsing in water. For the native sample, cocoons were collected from cells in the center of the nest, and care was taken to minimize contamination of the fraction with nest paper. Therefore, no peaks attributable to cellulose were observed in Fig. 4A. On the other hand, the purified sample was prepared from normal cocoons to which chewed wood fibers adhered. Nevertheless, no peaks of cellulose are apparent in Fig. 4B, indicating that all of the chewed wood fibers adhering to the cocoons were removed by purification. From this result, we can say that purification using aqueous LiBr solution was successful.

The main peaks were considered to come from Ala residues, because Ala is a major amino acid component of hornet silk, as shown in Table I. The peaks at 16.0 and 53.5 ppm were assigned to C_β and C_α carbon atoms, respectively, in Ala with an α-helix, whereas the peaks from C_β and C_α carbon atoms in Ala with a β-sheet appeared at 20.6 and 50.2 ppm, respectively (Asakura and Yao, 2002; Kricheldorf and Muller, 1984). Splitting of the peaks at 173 and 176 ppm was assigned to the C=O carbon atoms with a β-sheet and α-helix, respectively. The peaks of Ser C_α and C_β carbon atom with a β-sheet appeared at 55.9 and 63.8 ppm, and Ser C_α with an α-helix at 60.7 ppm, respectively (Asakura and Yao, 2002; Kricheldorf and Muller, 1984). The broad peak at 25 ppm was

interpreted as attributable to Leu (Kricheldorf and Muller, 1984). The Gly C_α peak appeared at around 43 ppm with low intensity.

The fact that peaks of C_α and C_β carbon atoms for Ala and Ser residues and the C=O carbon atom attributable to α-helix and β-sheet conformations were present (Fig. 4A) indicates the presence of both α-helix and β-sheet conformations in the native state of hornet silk. Recently, special attention has been focused on those silks, secreted by hymenopteran larvae, that exhibit distinctive conformations in comparison with other kinds of silk (Silva-Zacarin *et al.*, 2003). However, the details of the conformation of hornet silk have remained unknown until now. The coexistence of the α-helix and β-sheet may be characteristic of hornet silk and may be a distinctive conformation among the silk structures produced by spiders and other insects. Further discussion on the difference between the hornet silk and silkworm silk will be presented later.

Comparison of Figs. 4A to 4C reveals that the position of each peak is similar in each figure, indicating that not only the amino acid composition, but also the structural character of the coexistence of the α-helix and β-sheet in hornet silk were unchanged after purification and film formation. However, the peak intensities are different, indicating that the β-sheet to α-helix ratio was changed by processing: the β-sheet contents of the purified powder and HFIP film are higher than that of the native cocoon. Although the intensities found in ^{13}C CP/MAS experiments will not necessarily correspond exactly to the ratio of each nucleus, previous studies of silks have used ^{13}C CP/MAS experiments for quantification of the relative ^{13}C peak intensities (Yang *et al.*, 2004; Zhao *et al.*, 2003). Although the change in secondary structure estimated from the peak intensities of CP/MAS NMR spectra is not large, the thermal properties of hornet silk were significantly affected by this change in secondary structure, as described below.

FT-IR experiment

Expansion of the amide I region of the FT-IR spectrum of hornet silk in the native state is shown in Fig. 5A. It was already known that the IR bands at 1650 and 1620 cm^{-1} were attributable to the α-helix and β-sheet conformations, respectively (Chen *et al.*, 2001). Both of these peak components are apparent in Fig. 5A, indicating the coexistence of the α-helix and β-sheet in hornet silk in its native state. This result is consistent with the results of solid-state NMR experiments (Fig. 4A). Moreover, from Figs. 5B and 5C, it is apparent that the β-sheet contents of the purified powder and HFIP film are higher than that of native hornet silk, although these differences are only slight. These results were also consistent with those obtained from solid-state NMR experiments (Figs. 4B and 4C).

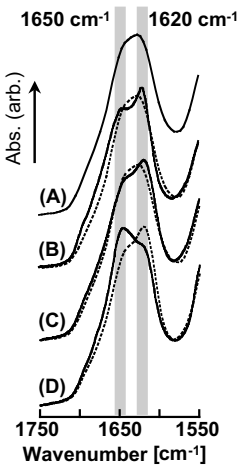

Fig. 5. Expansion of the amide I region of FT-IR spectra for silk of the hornet *Vespa simillima xanthoptera* Cameron; (A) hornet silk in the native state; (B) hornet silk after purification (solid line) and in the native state (dotted line); (C) film (solid line) and native state (dotted line) of hornet silk; (D) film of hornet silk before (solid line) and after (dotted line) rinsing in water.

Fig. 5D shows the FT-IR spectra of the film before and after rinsing in water. As described above (Fig. 2), residual HFIP solvent in the film after evaporation is removed during rinsing. From Fig. 5D it is apparent that marked conformational transition of the hornet silk from the α-helix to the β-sheet occurred during HFIP removal.

Differential scanning calorimetry (DSC) measurement

We investigated the thermal properties of silk of hornet *V. simillima xanthoptera* in the native state, purified powder, and film by DSC (data not shown). Each DSC curve displayed two endothermic peaks: one at around 80 °C was due to the loss of water, and the other was a result of thermal degradation. The temperatures of thermal degradation for native, purified powder, and film protein samples were 279, 297, and 300 °C, respectively; the temperatures of thermal degradation for purified powder and film were higher by about 20 °C than that of silk in the native state. As described above, the ^{13}C CP/MAS and FT-IR experi-

ments shown in Figs. 4 and 5 indicated that the β-sheet content of the purified powder and HFIP film were higher than that of the native state. This higher β-sheet content for the purified powder and HFIP film seems to be the reason for the higher thermal degradation temperature (Kweon and Park, 2001). On the other hand, there was only a subtle difference in thermal degradation between the purified powder and film; this was a result of the similar β-sheet to α-helix ratio for both morphologies, as shown by the ^{13}C CP/MAS spectra in Figs. 4B and C, and FT-IR spectra in Figs. 5B and C.

Comparison of hornet silk with silkworm silk fibroin

The β-sheet structure is a major feature of *B. mori* silk fibroin in the native-state (Kameda *et al.*, 1999a), whereas hornet silk consists of coexisting β-sheet and α-helix conformations. This difference in secondary structure could be largely attributed to the difference in solubility and thermostability between hornet silk and *B. mori* silk fibroin.

As described above, the native cocoon of *V. simillima xanthoptera* can be dissolved in HFIP at room temperature. In contrast, native-state silk fibroin of *B. mori* (the silk II form) is insoluble in HFIP solvent. When *B. mori* silk fibroin is dissolved in HFIP, alteration of the conformation from a β-sheet (silk II form) to a repeated β-turn (silk I form) structure is necessary before the fibroin will dissolve (Zhao *et al.*, 2003). These differences in dissolving ability of the hornet and silkworm silks in HFIP can be largely attributed to differences in β-sheet abundance. It can be said that the lower abundance of the β-sheet in hornet silk gives this silk an advantage for development as a material using HFIP as solvent in comparison with silkworm silks.

The β-sheet abundance in silk is also largely responsible for the silk's thermoproperties. *B. mori* silk fibroin in the native state, which has a higher content of β-sheets than hornet silk, has a higher thermal degradation temperature (330 °C) than does hornet silk (279 °C). As described above, the lower thermal degradation temperature for hornet silk is raised by processing.

B. mori silk fibroin with a 3_{10}-helix has been observed in HFIP solution; this structure is maintained in film prepared by drying from HFIP solution (Asakura and Yao, 2002; Zhao *et al.*, 2003). Additional treatment, such as methanol immersion or drawing, is needed to change the β-sheet structure in *B. mori* film. Our high-resolution ^{13}C NMR study of hornet silk revealed the presence of a helical or random structure in HFIP solution, where the peak of Ala C_β appeared at 14.7 ppm (data not shown). However, during the drying and water-washing process, structural transition occurred and the material returned to a coexisting structure consisting of α-helix and β-sheet, as shown in Figs. 4C and 5C.

SDS-PAGE analysis (Fig. 3) revealed that the silk of *V. simillima xanthoptera* did not consist of a single protein. Therefore, the amino acid composition of this silk (Table I) originates from a structural mixture. In contrast, *B. mori* silk fibroin predominantly consists of a single, so-called "heavy" chain protein. However, if hornet silk is used as a new natural material, it is worthwhile comparing the total amino acid contents of both silks, because their amino acid compositions could be largely responsible for their properties as materials. *B. mori* silk fibroin contains 46% Gly, 29% Ala, 12% Ser, 5% Tyr, and 2% Val (Kameda *et al.*, 1999a). Although it can be said that *B. mori* silk fibroin has a high content of Gly and few acidic residues, hornet silk has a low Gly content and high acidic residues. Because the amino acid composition of hornet silk differs from that of *B. mori* silk fibroin, hornet silk is likely to exhibit interesting properties as a natural material.

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