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Silk protein of the larval caddisfly, *Stenopsyche marmorata* (trichoptera: stenopsychidae)

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Abstract : *Stenopsyche marmorata* is one of the common caddisfly species in rivers and streams in Japan which is distributed in East Palaearctic Region. The larvae spin silk fiber between stones on the bottom of flowing water, the silk protein of *S. marmorata* would be one of the best sources of natural biopolymers that could be used as materials for aquatic industrial purposes. In order to analyze the basic characteristics towards further applications, we measured physical properties, thermal behaviors and molecular conformation of the silk protein of larval *S. marmorata*. The wet weight of the larval body and the silk gland of *S. marmorata* were 543.5±54.9 and 44.7±17.9 mg, respectively. The water content of the liquid silk protein in the silk glands of the living larvae was 73.4±2.2%. The elongation at the breaking point of the solid silk protein gland in dry state was 2.2%, while that of sample in wet state was 60.2%, suggesting the silk protein glands become soft and stretchable in wet conditions. FT-IR spectra of the silk protein film showed a major absorption band at 1650 cm^{-1} (Amide I band), which is attributed to the random coil molecular conformation. The silk fiber showed a major endothermic peak at 321°C on the differential scanning calorimetry (DSC) curve, which position is slightly higher than the endothermic peak for the silk film, suggesting an increase in the thermal stability for the silk fiber from caddisfly.

Key words: caddisfly, silk protein, fiber, elongation, film

Introduction

Most filter-feeding trichopterans that distribute commonly in a worldwide lotic system make a capture net by spinning a fine silk filament in order to gather their food, which also play some important role as removing suspended organic matter and heavy metals from the water (Cardinale et

al 2004; Wallece and Merritt 1980). The larval caddisflies (Trichoptera) seem to be one of the primary key species in aquatic systems. Actually, many studies have been conducted on the geometric aspects of the web structure in relation to the water flow, the uptaken organic matter, and the stabilized substrate (Cardinale et al 2004; Illes et al 2001; Loudon et al 1992). The silk protein of Trichoptera has drawn academic interest from the researchers who study silk proteins of silkworm because of the difference and comparison of fiber spinning mechanism. Yonemura et al (2006, 2009) reported the protein composition and the amino acid structure of silk proteins of caddisfly, and have compared it with those characteristics of Lepidoptera. The physical properties and the mechanism of spinning silk of caddisfly larvae have recently been studied (Brown and Ruxton 2004; Michalak et al 2005) from the scientific view of the expectation as new protein biomaterials, e.g., adhesion proteins secreted by blue mussel (Wiegmann 2005) and silk protein spun by spider (Vollrath 2000). Studies on more basic information for silk proteins from caddisfly are essentially important in order to make practical applications in the industrial fields. In this paper, we use the silk protein from larvae of *Stenopsyche marmorata*, which is one of the largest size among filter-feeding Trichoptera and comparatively easily collect in the midstream of Shinano River (Iizuka 1971). We conducted the basic researches on silk proteins by using the method that studied in Lepidptera, including infrared spectroscopy (FT-IR) differential scanning calorimetry (DSC) in order to analyze the basic characteristics of the silk proteins of *S.marmorata*.

Description of the Study Site

The Shinano River is the longest river in Japan. It stretches 367km with 11,900km^2 of drainage area, and after running through Nagano and Niigata Prefectures, flows finally into Japan Sea. The sampling site is located in the middle reaches near the Ueda city in Nagano prefecture, and a riffle which is mainly composed of cobbles and boulders often appears in that area (Fig.1). Under

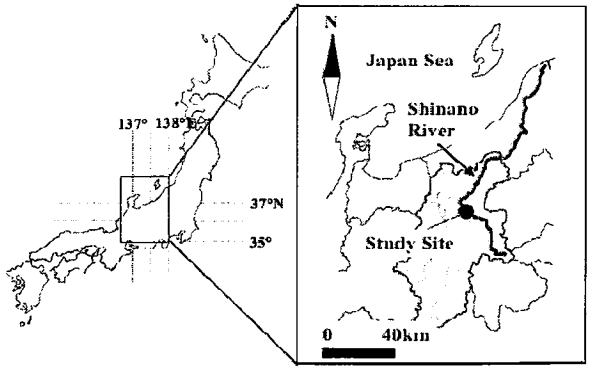

Fig. 1. Map of the study site.

riffles, we can frequently find many kinds of aquatic insects including *S. marmorata.*

Materials and Methods

Insect sampling and preparation

At the riffle in Shinano River, we collected the last instar larvae of caddisfly in May and June. The larvae of *S.marmorata* (Family Stenopsychide) were found under the substrates which spread over the whole of riffles. The larvae were brought to the laboratory in a plastic bag under cool conditions. For gathering the spun silk, the larvae were reared in the laboratory in the round plastic container (diameter 15cm × height 8.5cm) containing 6cm depth of tap water for a few days at room temperature (18℃ ~ 23℃). During the rearing, air flow was moderately supplied with an air pump. The silk fibers which were spun on the bottom of the container was collected from the container wall. We measured tensile properties and conducted DSC measurements for these fibers. We also used 2 other samples for the tensile test. One was the silk protein glands in dry state defined "solid silk protein gland" and the other was silk protein without glands which was mechanically drawn by hand defined "drawn solid silk protein". Silk film from caddisfly was prepared by casting liquid silk, which flows out of the silk gland on polystyrene substrate at room temperature. We conducted FT-IR analysis and DSC measuring for this silk film.

Measuring silk protein weight and water content

We measured the body weight of the caddisfly larvae and the wet weight of silk gland removed from the larvae by using analytic balance. The wet body weight and silk protein gland weight were measured subsequently. Silk protein glands were dried with 105℃ in hot air oven for 90 minutes. The water content of silk glands was measured before and after drying at 105℃ for 90 minutes.

Tensile properties

Tensile properties of the spun silk net, solid silk protein gland, and drawn silk gland protein were measured in dry state by using a Tensilon Model UTM-II-20, (Orientec Corporation Japan), using standard condition at 22 °C and 65% R.H. The sample length and the drawing speed were 30 mm

and 10mm/min, respectively. The tensile properties of the solid silk protein gland and drawn solid silk protein were measured in wet state (immersed in water for 15 minutes). Thus we determined the tensile strength and elongation at breaking point for the above samples.

FT-IR analysis and DCS measuring

Infrared spectra of silk protein from caddisfly were measured by using a FT-IR instrument Model IRPestige-21, (Shimadzu corpration, Japan). The Spectrum was obtained by making the average of 20 scans at a 4 cm^{-1} resolution. DSC was performed Rigaku Denki Co., Ltd. instrumental (model DSC-8230) at a heating rate of 10℃/min under N2 gas atmosphere from room temperature to 350℃. All the DSC curves were related to the first scan and sample weight was ca. 2mg.

Results and Discussion

Silk protein weight and water content

The average of the wet weight of the larval body and silk gland of *S.marmorata* were 543.5 ± 54.9 mg and 44.7 ± 17.9 mg, respectively. A significant positive correlation was observed between the wet weight of body and silk gland (Fig.2). The water content of the liquid silk protein extracted from the silk glands of the living larvae was 73.4±2.2%.

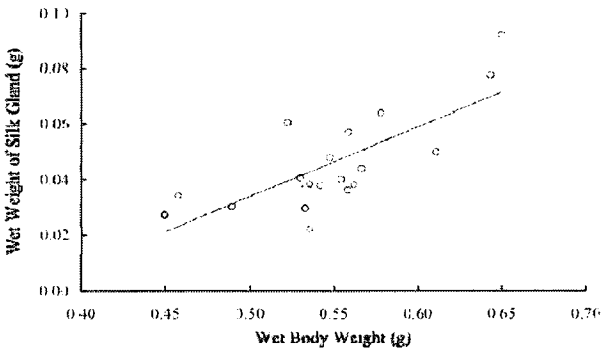

Fig. 2. Wet body weight vs. wet silk gland weight.

The wet body weight of the *S.marmorata* larvae was closely similar to the data reported by Aoya (1987). Obtaining comparatively a large amount of silk protein is one of the distinctions of using the larvae of *S.marmorata* as the experimental insect. The water content in the silk gland of the silkworm is around 30% (unpublished), suggesting that the silk protein content in the silk gland of the larvae of *S.marmorata* is more low concentration. These data show that caddisflies spin the water-insoluble silk protein in the water by using a low concentrated silk solution.

Tensile properties

Fig.3 shows the tensile properties of samples from caddisfly. The elongation of the solid silk protein gland and drawn solid silk gland protein in dry state was about 2-3% at breaking point (Fig. 3.a.c.). It is interesting to note that the elongation of the solid silk protein gland in wet state was 60.2% (Fig. 3.d). The drawn solid silk protein in wet state cannot be carried out because it dissolved into the water during preparation because of the lack of silk gland and the low degree of orientation. These results suggested that the silk protein of *S. marmorata* larvae in dry state is bristled and it become soft and stretchable in wet state. Concerning the tensile strength, the resemblance between the spun silk net fiber (Fig. 3.b) and the solid silk protein gland (Fig.3.a) in their dry state is remarkable, but the elongation at break of the spun silk net fiber is higher than the other one. After showing the maximum tensile strength, spun net fiber showed gradually decreased tensile strength (Fig.3b).

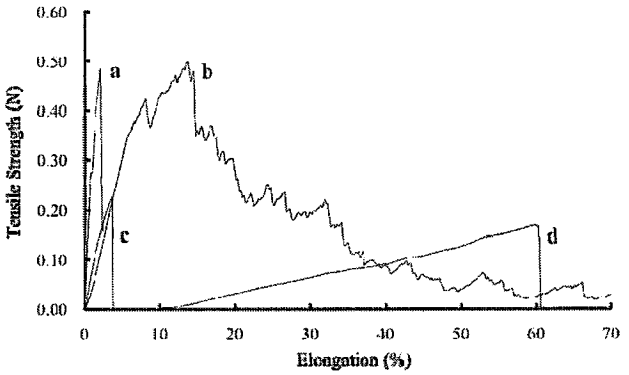

Fig. 3. The strength and elongation curves of several silk protein materials. (a) Solid silk protein gland. (b) Spun silk net. (c) Drawn solid silk protein without gland. (d) Solid silk protein. a, b, and c were measured in dry state, d was measured in wet state.

FT-IR analysis and DSC measuring

Fig.4 shows the FTIR spectrum of the silk protein film, displaying intense absorption bands at 1635 cm^{-1} (amide I), 1515 cm^{-1} (Amide II), and 1235 cm^{-1} (Amide III), assigned to the β -sheet conformation (Freddi et al 1995, Shao et al 2005). In addition, minor amide bands at 1640, 1650, and 1657 cm^{-1} are attributed to the random coil conformation (Shao et al 2005; Teramoto et al 2003).

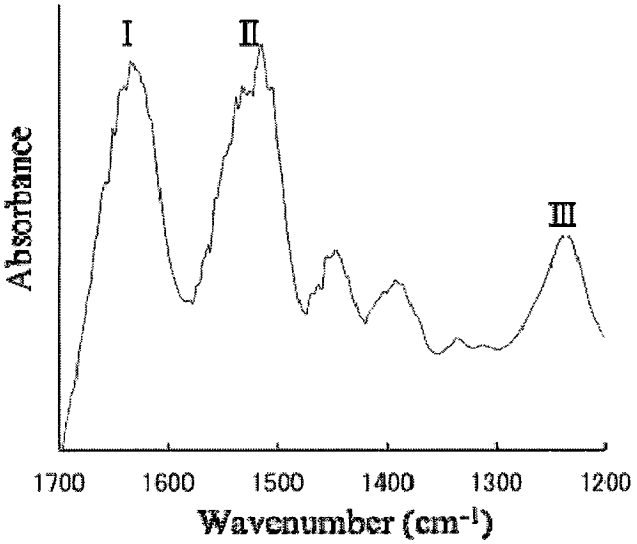

Fig. 4. Infrared spectrum of the silk protein film.

These results suggested that the conformation of silk protein film from silk glands of larval *S.marmorata* has a random coil conformation with a small amount of β -sheet structure.

Fig.5 shows the DSC curves of the spun net and silk protein film. The endothermic peak appeared at 231℃ for the silk protein film (Fig.5a), while the DSC curve of the spun net (Fig.5b) showed the endothermic peak at 321℃. These data imply the thermal decomposition of the spun net occurs around 320℃ and the silk protein film softens around 231℃. The position of the decomposition temperature of the spun net is similar to that of silk fibers from the larvae of B. mori, silkworm (Tsukada et al 1991), regardless of the different amino acid composition and tensile properties between silk fibers from aquatic insects and silkworms (Brown and Ruxton 2004; Park et al 2003; Yonemura et al 2006).

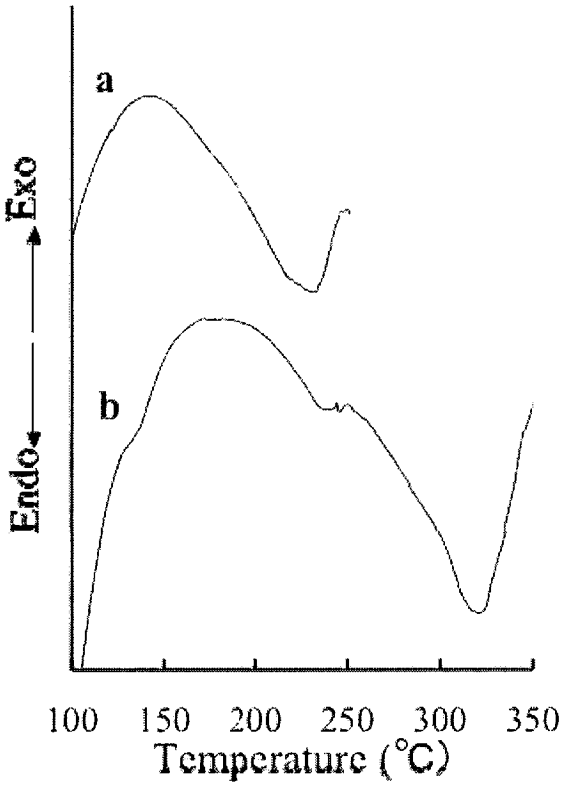

Fig. 5. DSC curves of silk protein film (a) and spun net fiber (b)

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