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Isolation of Silk Proteins from a Caddisfly Larva, Stenopsyche Marmorata

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Abstract

The protein composition of the silk gland of caddisfly larva, *Stenopsyche marmorata* was investigated. Dissected silk glands of the larvae were treated in a Tris buffer (pH 8.0) containing 8 M urea, and the proteins in the lumen of the silk gland were solubilized by freeze and thaw. The major proteins of the silk gland were precipitated upon dialysis against diluted acetic acid. The precipitate was dissolved in a basic Tris buffer containing high concentrations of urea and dithiothreitol. Trypsin-like proteinase activity was detected in the silk gland extract. Therefore, all extraction procedures were re-visited in the presence of Leupeptin, which is a specific inhibitor of the trypsin-like proteinase in the silk gland. The improved preparation of the silk gland extract contains a high molecular weight protein, namely *S. marmorata* silk protein-1 (Smsp-1), of which the molecular weight was estimated as 310–320 kDa on the basis of electrophoretic mobility on cetyltrimethylammonium bromide-poly (acryl amide) gel electrophoresis. Subsequent gel filtration exhibited a chromatogram, where the Smsp-1 bands were found from void (ca. 2000 kDa) toward the 300 kDa region, latter of which corresponds to the estimated molecular weight of monomeric Smsp-1. The Smsp-1 eluted near void region was found to be electrophoretically homogenous, suggesting that Smsp-1 molecules associate in a multimeric form. Sodium dodecylsulfate-PAGE indicated that the fractions eluted at the 650–280 kDa region also contain Smsp-1, together with three kinds of low molecular weight proteins, which were designated as Smsp-2 (26 kDa), -3 (21 kDa), and -4 (16 kDa). These results suggest that the Smsps-1, -2, -3, and -4 can form a larger complex to be spun into net threads.

Keywords: *Stenopsyche marmorata*; Silk gland proteins; Protein composition analysis; Isolation

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1 Introduction

A Stenopsychidae caddisfly, *Stenopsyche marmorata*, forms a relatively major habitat in aquatic insects around the middle reaches of the Chikuma river, Nagano Prefecture, Japan [1, 2]. These species has been investigated from multiple research aspects in environmental science for their roles in aquatic ecosystems, as nuisance species in urban activities [3], and more recently in connection with active biomonitoring for trace metals in the environment [4]. Trichoptera was also found to be a useful indicator organism for environmental pollution [5]. Another caddisfly species, Hydropsychidae, has been reported to form a tremendous build-up of larval net on the inner walls of penstocks of hydroelectric facilities in Japan, which reduces water flow inside the penstocks, resulting in decreased electricity generation [6]. Hence, the life cycles of caddisflies are still closely related to the present issues in compatibilities between environmental conservation and the economy in human society. Therefore, substantial research works on the caddisfly in given aquatic systems have been required, especially in regard to environmental biology. In this sense, Hirabayashi *et al.* described the seasonal distributions of adult and larval caddisflies in the middle reaches of the Chikuma River [1], and these works can contribute to prediction of the massive flight seasons of the adult caddisfly [2].

From another point of view, a major aspect of the issue of caddisfly larvae as an aquatic nuisance species is their settlement behaviors. Caddisfly larvae are well known as net-spinning aquatic insects, of which the net threads can tightly attach to underwater substrates, such as rocks and stones, to entrap their nutrients. The net threads of caddisflies are occasionally referred to as "caddis silk" since they originate from the secreting organ, *i.e.*, the silk gland. The net threads are proteinaceous fibers and the biosynthesis of the precursor proteins occurs in the silk gland, where the net thread precursor proteins are stored at very high concentrations in the lumen of the gland. Beams and Sekhon made one of the earlier studies on the secretion of the net thread proteins from the *Platyphylax designuts* silk gland cells into the gland lumen [7]. However, even to date, very restricted numbers of research publications on the settlement of caddisfly larvae are available, especially for the molecular mechanism on spinning and adhesion of the net thread proteins.

Yamamoto *et al.* first described the seasonal transition of the amino acid compositions in the nest and silk gland of *Stenopsyche griseipennis* (synonymy, *S. marmorata*) and the bonding strength of the silk gland contents on various solid surfaces [8]. As for the protein composition in the silk gland of a caddisfly larva, *Pycnopsyche guttifer*, Case *et al.* reported that the silk protein has a molecular weight of ca. 800 kDa, based on electrophoresis, and also that there was no protein directly extractable from the net [9]. More recently, a molecular biological study has been done by Yonemura *et al.*, and they found that the silk proteins from two different caddisfly suborders, *Hydropsyche angusipinnis* and *Limnephilus decipens*, contain similar amino acid sequence motifs but lack poly(alanine) and poly(glycylalanine) motifs. The latter are often found in lepidoptran and spider silks [10]. Since there have been few research works on the biochemical analysis of the net thread proteins in the silk gland, the molecular structural properties of caddisfly silk proteins tend to be understood as the counterparts of those in the silkworm *Bombyx mori*. On the other hand, there has been no direct evidence that aquatic Trichoptra and terrestrial Leidoptera adopt the same mechanisms to spin proteinaceous threads, even though their living environments are completely different from each other.

Therefore, to explore the net-spinning and adhesion mechanisms of the caddisfly larva at molecular levels, protein composition analysis and isolation of the net thread proteins are essential