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A Repetitive Secretory Protein Gene of A Novel Type in *Hydropsyche* sp. Is Specially Expressed in the Silk Gland

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Objectives

Trichoptera, or caddisflies, comprise one of the major aquatic insect orders. Like Lepidoptera, caddisflies are capable of spinning silk from specially modified salivary glands, and the diversity of ways this silk is used probably accounts for the success of the order as a whole. These utilize silk to construct both larval and pupal shelters, often incorporating materials from the environmental among the silk thread. In this study, we try to find and characterize novel type genes that should be translated to major component protein of aquatic silk.

Material and Methods

Material – Insect : Late larval Trichoptera *Hydropsyche* sp.

Methods – Hematoxylin & Eosin Staining

Expressed Sequence Tags Analysis

Northern blotting & Dot blotting

in situ hybridization

Recombinant protein from GST fusing

Results and Discussion

In this study, we find new values of the genes expressed in *Trichoptera*. 397 cDNA clones were sequenced from 5' end of the cDNAs. Cluster analysis identified 48 groups of sequences and 206 singletons indicating that the database represents a total of 249 genes. Putative functions say that 101 ESTs(26%) matched known genes, while 295 ESTs did not match with any known genes. Among the redundant ESTs, High-copy ESTs containing ten or more ESTs compose 42% of total ESTs. Northern dot blotting showing silk gland specificity and intensity represent that 106 ESTs were expressed in the silk gland. Finally collected EST, SG0165, is base on their abundance and specificity for the silk gland. The result of SG0165 EST examined by Northern blot show that it has two transcript, 1300 bp and 1800 bp length, and it is expressed in the silk gland specifically. Each of two transcripts is named Nf-1 and Nf-2 respectively. Nf-1 and Nf-2 have repetitive sequence and highly conserved sequences. As a result of *in situ* hybridization, those are more expressed in the posterior lobe and outer layer of silk gland than the anterior lobe and inner layer. We suggested that two genes were translated to major component proteins of

aquatic silk and these proteins were secreted to the lumen of silk gland. To find whether these genes were translated to secretory proteins or not, we constructed recombinant protein of Nf-2 gene using GST fusion protein system. This recombinant protein was being used to make polyclonal antibody to detect intact silk protein of *Hydrosyche* sp. by western blot. These results will suggest that Nf-1 and Nf-2 are novel type of genes and should be the major component genes of aquatic silk of Trichoptera

Nf-1 Repetitive Amino acid Sequence

1st repeat CCQHPRYDRCGVFGHHLWDYVKYSASWSHSVENY
2nd repeat HGVPHGNYYYPLWNYCCQHPRCNRCGIFGHHLQDYVKYSASWSHSVEDY
3rd repeat FYWDDSWVHGVPHGNYYYPLWNYCCSHPRCNRCGIFGHHLQDYIKYSASHSVEVE

Nf-2 Repetitive Amino acid Sequence

1st repeat CCQRPYDGRCGIFGNHLWDYVKYSASWSHSVENY
2nd repeat HGVPHGNYYYPLWNYCCQHPRCNRCGIFGHHLQDYVKYSASWSTSVENY
3rd repeat FYWDNSWVHGVPHGNHYYPLWNYCCQHPRCNRCGIFGHHLQDYIKYSASWSTSVENY
4th repeat FYWDDSWVHGVPHGNHYYPLWNYCCHHPRCNRCGIFGHHLQDYVKYSASWSTSVEDY
5th repeat FYWDDSWVHGVPHGNCYYPLWNYCCSHPRCNRCGIFGQHLWDYVKYSASHSVEVE

Figure 1. Deduced repetitive amino acid sequences are highly conserved. Not only Nf-1 but also Nf-2 has a same unit of repetitive amino acid sequences. The longest and shortest repetitive amino acid sequences were composed of 57 and 34 amino acids respectively. The shortest one was the core sequence and the others were branched out. Green letters mean conserved amino acids.

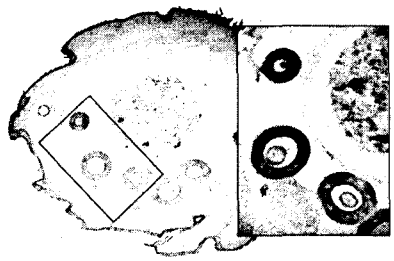

Figure 2. *In situ* hybridization of DIG labelled Nf-1. This gene was expressed in silk gland overall. But it was more expressed in the posterior silk gland than anterior and outer layer of silk gland than inner layer.

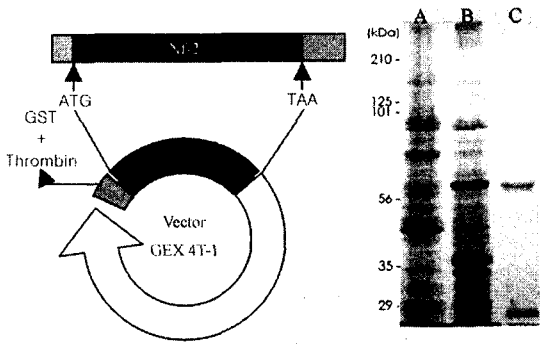

Figure 3. Recombinant protein by using GST fusion protein system. Left panel shows the vector system of GEX 4T-1 and insert of Nf-2 gene. Right panel shows the expression and isolation of recombinant protein. A and B show insoluble material of inducted and sonicated *E.coli* and supernatant of it respectively. C indicate that purified recombinant protein's size is about 62kDa (GST: 29 kDa, Nf-2 protein: 33kDa).

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