

STRUCTURAL STUDIES ON SILK PROTEIN FIBRE FROM PSEUDOSCORPION

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ABSTRACT

Pseudoscorpion species were collected from Western Ghat's forest habitat. They were identified as *Chelifer* sp. of order *cheliferoidea* to the class *Arachnida* (Mahnert, 1991). The silk gland of chelicerae was dissected out. The samples of the fibroin protein secreted from the silk gland were subjected for physical, chemical and biological analysis. It was found that false scorpion fiber could be dissolved in 6N HCL / 50% propionic acid medium. The amino acid composition was recalled the blend of amino acids present in the fibrous protein of spider and *Bombyx mori*. The structural studies suggesting collagenous in nature and a relationship with silk fibers of spider and silk insect. The results are discussed with earlier literature.

Key words : Pseudoscorpion, chelicerae, structural studies, silk fibers of spider and silk insect.

INTRODUCTION

Pseudoscorpion is also described as false scorpions. They are very similar in structure as that of true scorpion except in the absence of tail with poison sting. In general the size of this arachnid species is very small. It ranges from two to eight mm and has dorso-ventrally flattened body.

The large pedipalps have venoms glands. Pseudoscorpion also has chelicerae. They use chelicerae for break down the food and spin silk like protein fiber web (Weygoldt, 1969). They have an extended life cycle of one to three years. The biology of life cycle is interesting. The male produces spermatophore and pulls the female over it during the mating dance. The female carries a silk and egg pouch of twelve to fourteen eggs on her abdominal segments about three weeks. The hatched brood releases young ones

which looks like an adult. The young ones undergo three moultings before becoming adults (Proctor Heather, 1993).

Pseudoscorpions develop silken cocoons during winter. They are very active in summer. They feed small insects. They are considered as beneficial arachnid. They never bite a human. The most biological significant character is they use the legs of other organisms for the locomotion. It is described as "phoresy" of pseudoscorpion. Normally they use legs of "Daddy long legs" for their movements. The biology of pseudoscorpion is available at www.biology.ulberta.ca/bsc/new24_1/pseudoscorpions.html.

In this present studies pseudoscorpions are collected from the shrub jungles of Western Ghats, India while preparing insect biodiversity

of this region. The collected species are identified as *Chelifer* sp. of order *cheliferoidea* belongs to the class *Arachnida* (Mahnert, 1991). An attempt has been made in this present investigation on the physical, chemical and biological properties of

MATERIALS AND METHODS

The materials used for the present investigation were the false scorpions (Fig.1) and their silk gland secretory protein fiber. They were collected from shrub jungles of Western Ghats forest regions of Southern India. The specimens



Silk samples (glandular / reeled fiber) were kept at room temperature. The silk glands were dissected out through incision by observing under microscope. They were immediately placed in a watch glass containing 0.01M sodium chloride and 0.015M sodium citrate buffer to inhibit protease released by the pseudoscorpion. The fiber proteins were subjected for the solubilization, hydrolysis and amino acids analysis as adopted by Lombardi and Kaplan (1990). The methodology is explained as below.

Solubilization

Solubility of the fibrous protein understudy was carried out with various solvents at room temperature. The list of solvents used for the analysis is given in Table 1.

Amino acid analysis

The Electrophoresis and Amino acid analysis were performed as out lined in Gordon (1970).The

the silk protein of pseudoscorpion. The understanding of pseudoscorpion silk protein structure is worthwhile and may aid in indentifying naturally occurring bio-fibers.

were dehydrated by alcoholic grades, stained and mounted for species identification. The chelicerae were dissected out from living animals (Fig.2). The secretion from the silk gland was collected in glass tubes. The reeled samples from the secretion of glands were also obtained.

fibrous protein *in question* was hydrolyzed by boiling with 6N HCl. The hydrolyzed protein was vacuum dried in Borosil vials. Then the sample was dissolved in 2 ml of HCl/Propionic acid (1:1, v/v) mixture at room temperature. Amino acid analysis was done by conventional two dimensional paper chromatographic method. The irrigation fluid was Butanol/acetic acid/water (1:2:1). Ninhydrin was used as developer. A standard amino acid chromatogram was prepared with known samples for comparison. Each identified amino acid was admitted for the densitometric analysis for the determination of molar concentration.

RESULTS

The structural studies on chelicerae of a pseudoscorpion was disclosed the following details. The photomicrograph and the drawings of the dissected out chelicerae *in situ* silk gland are

given in figures 2 and 3 respectively. The gland was a bag like structure with many flagellums on the left side. There were three chitinous finger like appendages in the bottom. In the upper part there was a branched chitinous tubercle. There

was an evidence for the secretion of fibrous thread like protein from the branched tubercles. Underneath to this tubercle the slender bag like gland was present (fig 3).

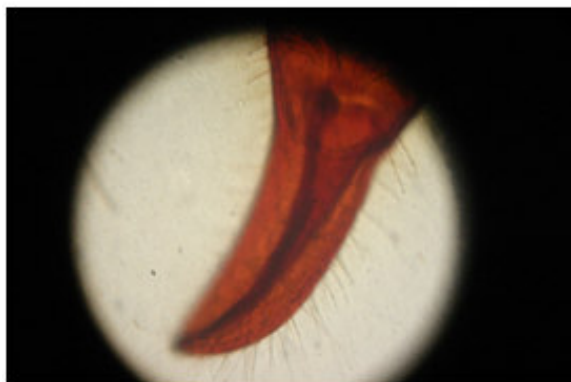


Figure. 2

The secretion from the silk gland was collected in sterilized vials. It was subjected for the solubilization and amino acid composition analysis.

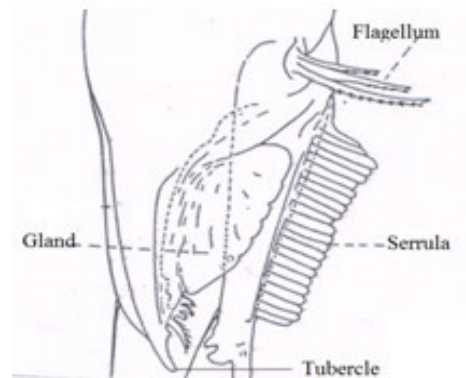


Figure. 3

Table 1. Solubility of pseudoscorpion protein fiber in different solvent systems.

Sl. NO	Solvent	Solubility State at Room Temperature*
1	Water	in soluble
2	1 N HCl	in soluble
3	2 N HCl	in soluble
4	3 N HCl	in soluble
5	4 N HCl	in soluble
6	5 N HCl	partially soluble
7	6 N HCl	partially soluble
8	1 N KOH	in soluble
9	Chloroform	in soluble
10	Absolute alcohol	in soluble
11	8M Urea	partially soluble
12	50 % lithium bromide	partially soluble
13	1% SDS	in soluble
14	5% Mercaptoethanol	in soluble
15	6 N-HCL/50 % Prop ionic acid	Soluble

* 28 – 31 Degree Celsius.

Table 1 is furnished with the results of solubilization test with various solvent. From the data it was evident that false scorpion's fibroin

could only be totally dissolved in hydrochloric acid and propionic acid (1:1) at 60 ° C for few min. (less than 10 min.). On the other hand the

silk protein of the pseudo scorpion *in question* was failed to dissolve in solvents such as water, 1N HCl, 2N HCl, 3 N HCl, 4 N HCl, 1 N KOH, chloroform, absolute alcohol, mercapto ethanol and SDS. Besides there was a partial solubilization in the cases of solvents such as 8 M urea and 50 % Lithium bromide (Table 1).

The above results would suggest that medium containing 6N HCl / 50 % propionic acid (1:1, v/v) may be a good solvent for the fibrous protein under study.

Fig 4 is the photograph of the chromatogram developed from the analysis of amino acids present in the silk of pseudoscorpion.

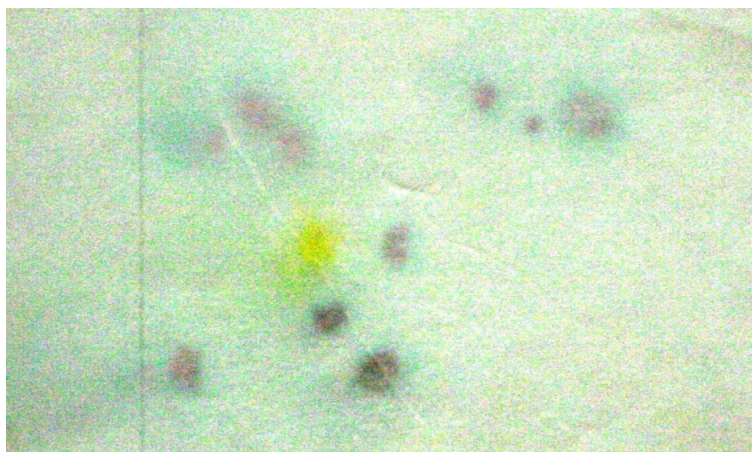


Figure 4. AMINO ACID CHROMATOGRAM OF SILK PROTIEN

There were eleven amino acid spots on the chromatogram. The amino acids were identified by the standard chromatogram run with the known samples of amino acids. This discloses the presence of Glycine, Alanine, Isoleucine, Serine, phenyl alanine, valine, glycine, threonine, histidine, lysin and glutamic acid. Several similar such chromatograms were prepared with the samples. The amino acids were pooled separately. The molar concentration of the amino acids was determined with the help of densitometer.

Table 2 is furnished with the data of 11 amino acids drawn from the chromatographic

studies. the glycine had the maximum concentration of 31.5%. Next to glycine the alanine has 20.1%. The amino acid isoleucine was estimated as 19.1%. From the above results it would be reasonable to suggest the silk of pseudoscorpion is collagenous nature. The table 2 is also provided with amino acid composition of spider fibrous protien and the silk protien of *Bombyx mori*. The comparative observations in the compositions of amino acid would suggest a structural analogy with other fibrous silk proteins of biological origin.

Table 2 . Amino acid composition of protein fiber of pseudoscorpion with spider and silk insect.

Amino acid	pseudoscorpion	Spider [@]	Silk Insect ^s
Glycine	31.5	37.1	41.1
Alanine	20.1	21.2	29.7
Iso Leucine	19.1	11.7	3.6
Serine	10.3	4.5	12.4

Threonine	8.6	1.7	1.2
Glutamic Acid	5.8	11.7	3.6
Phenyl alanine	2.9	10.2	7.5
Lycine	1.1	1.0	-
Histidine	0.6	1.1	-
Valine	0.2	11.7	3.6

@ Data on *Nephila clavipes* silk fibroins (Lombardi and Kapalan, 1990).

\$ Data on *Bombyx mori* silk fibroins (Lucas et al, 1955).

DISCUSSION

It is a challenging work that studies to be carried out on structural proteins like silk, collagen, elastin, resilin and keratin without damaging the protein polymer. The results of present investigation show that silk proteins of spider and insect origin have a relationship with the protein fiber of pseudoscorpion. Like other silk proteins, pseudoscorpion fibrous protein does not solubilized in the solvents given in table 1. However it can be solubilized in strong acid (6 N HCl / propionic acid). Besides the observation of single homogeneous band in gel electropherogram even after solubilization suggesting the disintegration of protein polymer. Similar such properties have also been observed by the silk fibers of spiders and insect (Lucas et.al., 1960 and Anderson,1970).

The strong acid solvent is said to be oxidizing agent. The results of amino acid composition reveal the loss of sulphur containing amino acids. This would suggest that – SH/-S-S- linkages do not affect the cross linking of fibrous proteins are the integrity of silk protein polymer of pseudoscorpion. This corroborates the earlier findings of spider silk structure by Lucas and Kaplan (1990).

The high content of the glycine and alanine residue would suggest that these amino

acids may provide a significant role in preserving the structure of silk. Similarly the insolubility of silk fiber in Urea, Lithium Bromide and SDS would assume that fibroin structural stability is not only due to hydrogen bonding. It also discloses the existence of some other specific chemical bonding. Seifter and Gallup (1966) have postulated that the structure of silk fiber may consist of multiple protein regions with specific chemical linkages involving covalent and non covalent interactions.

The comparative amino acid composition of pseudoscorpions, spiders and *Bombyx mori* have shown a uniform nature in chemical framework. The assessment and interpretation of data would suggested that there is no significant variation between the silk proteins of the other organisms. Therefore it is reasonable to assume that pseudoscorpions chelicerae gland silk fibroin shows structural homology as that of *Bombyx mori* silk . Further the present studies substantiate the information of silk fibroin protein studied in other pseudoscorpion species called *Neobisium maritimum* (Hunt 1970).

It is desirable to further characterize these results by high defined analytical procedure and X-ray diffraction studies. The distinction of pseudoscorpion silk genes, gene sequences, studies on cloning these genes in microbes may give benefit of using this naturally occurring fiber.

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