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Biochemical Study of Pyriform Silk of Spider *Nephila pilipes*

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Abstract

Female *Nephila pilipes* constructs a huge orb web between tall trees. Nearly rectangular, uniform and narrow mesh in capture area of *Nephila pilipes* web seems to be more efficient at intercepting prey. Pyriform silk is used as glue to form bonds between separate threads for attachment points. Nature evolved this well-designed adhesive before thousands of years which definitely provide important insights in designing new synthetic adhesives. The main goal of this study is to understand the biochemistry of this natural adhesive. In the present work, biochemical analysis of pyriform silk of *Nephila pilipes* was performed with the help of FTIR spectrum. The FTIR Spectrum of spider silk provided strong bands of photosensitive amide I, II and III regions. It also revealed the presence of different functional groups between amino acid residues in spider silk biopolymer. Spectrum confirmed presence of β sheets, β turns, random coil and α helix. These structures give typical outstanding mechanical properties to silk threads.

Keywords: *Nephila pilipes,* Spider silk adhesion, pyriform silk, biomaterial.

Introduction

Spider webs have attracted human attention for centuries. The fundamental unit of behaviour in the orb web spiders is the construction and design of the web [1]. Female *Nephila pilipes* constructs a huge orb web between tall trees. The main structural elements of an orb web of *Nephila pilipes* are the bridge line, the frame thread, the radii and the sticky or capture spiral. Each junction in the huge web is joined with a sliding connections i.e. attachment disc form by pyriform silk.

Spider attachment disc silk fibers are spun into a viscous liquid that rapidly solidifies, gluing dragline silk fibers to substrates for locomotion or web construction [2]. Attachment discs spun from pyriform silk fibers allow spiders to move safely from place to place while secured to a dragline and to attach their webs to a variety of surfaces. Wolff *et al*. [3] described an intriguing example of the use of attachment silk for prey immobilization that comes with the costs of reduced silk anchorage function, increased pyriform silk production and additional modifications of the extrusion structures i.e. spigots to prevent their clogging.

The outstanding mechanical properties of spider silk have motivated to study the primary and secondary structure of pyriform silk proteins. The infrared spectrum of protein provides a wealth of information on structure and environment of the protein backbone and of the amino acid side chains [4]. It is a useful and popular tool for investigation of protein structure.

Materials and Method

Pyriform silk samples from attachment disc of the orb web of *Nephila pilipes* were taken. Spectrum of this silk was obtained by using SHIMADZU Fourier Transform Infrared Spectrophotometer, Model No. 8400S in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1} for 20 scans. Image analysis was performed with IRsolution software version 1.40.

Result and Discussion

The infrared spectrum of protein provides a wealth of information on structure and environment of the protein backbone and of the amino acid side chains [4]. FTIR spectrum of attachment disc silk of *Nephila pilipes* showed number of well defined peaks in the range of 600-1800 cm^{-1} (Fig.-1). Possible assignments for amino acid side chains are given in table -1. The IR Spectrum of silk provided strong bands of photosensitive amide I, II and III regions. *Nephila pilipes* pyriform silk showed a distinct amide I band in the range of 1600-1700 cm^{-1}. Peaks between 1500-1600 cm^{-1} indicates amide II region. The peaks from 1200-1470 cm^{-1} comes under amide III region, resulting from mixture of several coordinate displacements. The FTIR spectrum reveals the presence of different functional groups like C=O, C=C, N–H, C–H, C–N, CH_3, COOH, COH etc. These are between amino acid residues in silk peptide polymer which gives rise to well known signature in the Infrared region of the electromagnetic spectrum.

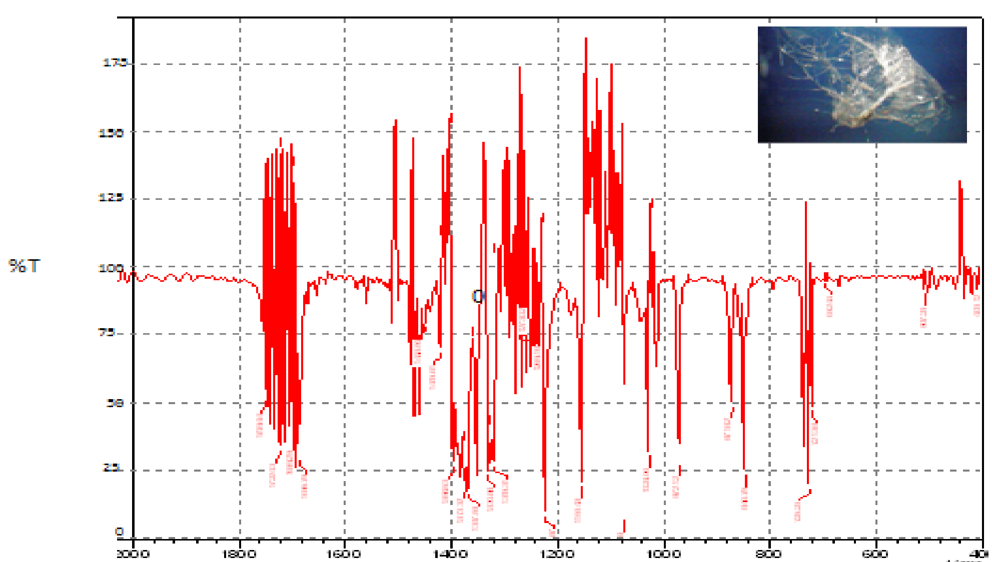

Fig. 1: FTIR spectrum of pyriform silk of *Nephila pilipes*

Table 1: Table showing amino acids side chains in FTIR spectrum of pyriform silk of *Nephila pilipes*

Observed values (cm^{-1})	Assignments	Literature values (cm^{-1})
1749 & 1722	Glu, ν (C=O)	1712-1788
1699	Asn, ν (C=O)	Upto 1704
1685	Gln, ν (C=O)	1659-1696
1448	Alanine	1447,1453*
1419	Pro, ν (CN)	1400-1454
1394	Asp, ν_s (COO^-)	1392-1425
1373	δ (CH_3)	1375
1367	δ (CH_3)	1368
1330	Trp, ν (CN)	1334
1319	Trp, δ (CH)	1315-1350
1273 & 1269	Tyr, ν (CO), ν (CC)	1267-1277
1222	His, ν (CN); Ser, δ (COH)	1223, 1181-1420
1153	Asp, Glu, ν (C-O)	1120-1253
1076	Thr, ν (CO)	1075-1150
1028	Gly- Gly Sequence	1015±10*
972	Ala-Gly Sequence	975*
871	Thr, δ (CO^2H)	865-942

Table-2: General assignments for secondary structure elements to characteristic wave numbers measured by FTIR spectroscopy

Secondary Structure Assignments	Observed Value (cm^{-1})	Literature Value (cm^{-1})
β sheet	1699	1690
β turns	1685	1660-1685
α helix	846,871	643-967
Random Coil	1269	1261

The FTIR spectrum of silk showed the presence of various amino acid side chains like alanine, glycine, glutamic acid, aspartic acid, tyrosine, proline, trypotphan, serine and histidine etc. [4,5]. FTIR spectrum also confirmed presence of β sheets, β turns, random coil and α helix (Table -2). The peaks near 1690 cm^{-1}

confirms presence of β sheet structure. Whereas, the peaks in the range of 1660-1685 cm^{-1} indicate presence of β turns. The absorption of IR rays at 1261 cm^{-1} is associated with random coil formation. Spectra shows well defined peaks in the region of 844 to 873 cm^{-1}. These lower frequency wave numbers in the range of 643-967 cm^{-1} shows absorption peaks for α helices.

In table, the amino acids are abbreviated with the three letter code; δ- In plane bending vibration; ν- stretching vibration; ν_s - symmetric stretching vibration; ν_{as} - asymmetric stretching vibration; γ_r = rocking vibration; Values for band position are taken from Barth [4] and Zemlin [5].

A great deal of science and technology is involved in this simple action of bonding. Proper adhesion of sliding attachments by pyriform silk influences the long term functionality of the web. The softness and the extensibility of hundreds of pyriform fibers attaching the dragline threads combined with low peeling angles contribute to the strength of these attachment discs [6]. However, the mechanical performance of natural pyriform silk fibers is unknown because of its tiny structure and the difficulty in collecting it. The FTIR spectrum of pyriform silk showed number of peaks that are characteristics of specific types of molecular vibrations. The spectrum of silk revealed the presence of majority of amino acid side chains linked in the polypeptide chains. Percentage of alanine, glycine and proline affect the degree of crystallinity, helicity or amorphous structure [8] and elasticity [7]. Silk protein or spidroin largely consist of repeated sequence of amino acids. These repetitive regions of the spidroin are confined in highly organized secondary structure which enhances the strength of spider silk. The peculiar properties of spider silk may be due to presence of secondary structural elements like β-sheets, β-turns, α-helices etc. The amino acid composition of PySp1 is obviously distinct from other spidroins. The sequence of *Araneus ventricosus* silk by Wang *et al.* [9] made an important contribution to understand pyriform silk protein structure and also provides a new template for recombinant pyriform silk proteins with attractive properties.

Conclusion

The adhesives used in spider webs evolved over millions of years into a class of natural materials with outstanding properties. The primary protein structure of pyriform silk is composed of a sequence of amino acid residue which is responsible for the defining molecular structure. The secondary protein structure like β-sheet, β-turns and α helices etc. are the key elements of this wonder fiber, responsible for its outstanding properties. *Nephila pilipes* build an aerial trap in the form of orb web. Attachment discs made from pyriform silk increase the strength of orb web immediately post-construction.

Thus, orb web spider *Nephila pilipes* produces high quality bio- adhesive in the form of attachment disc of pyriform silk. The detail study of the biochemistry and processes used by spider will provide many insightful strategies of designing new adhesives.

Conflict of Interest: The authors declare no conflict of interest

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