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Structural and optical studies on selected web spinning spider silks

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ABSTRACT

This study investigates the structural and optical properties in the cribellate silk of the sheet web spider *Stegodyphus sarasinorum* Karsch (Eresidae) and the combined dragline, viscid silk of the orb-web spiders *Argiope pulchella* Thorell (Araneidae) and *Nephila pilipes* Fabricius (Nephilidae). X-ray diffraction (XRD), Fourier transform infra-red (FTIR), Ultraviolet-visible (UV–Vis) and fluorescence spectroscopic techniques were used to study these three spider silk species. X-ray diffraction data are consistent with the amorphous polymer network which is arising from the interaction of larger side chain amino acid contributions due to the poly-glycine rich sequences known to be present in the proteins of cribellate silk. The same amorphous polymer networks have been determined from the combined dragline and viscid silk of orb-web spiders. From FTIR spectra the results demonstrate that, cribellate silk of *Stegodyphus sarasinorum,* combined dragline viscid silk of *Argiope pulchella* and *Nephila pilipes* spider silks are showing protein peaks in the amide I, II and III regions. Further they proved that the functional groups present in the protein moieties are attributed to α-helical and side chain amino acid contributions. The optical properties of the obtained spider silks such as extinction coefficients, refractive index, real and imaginary dielectric constants and optical conductance were studied extensively from UV–Vis analysis. The important fluorescent amino acid tyrosine is present in the protein folding was investigated by using fluorescence spectroscopy. This research would explore the protein moieties present in the spider silks which were found to be associated with α-helix and side chain amino acid contributions than with β-sheet secondary structure and also the optical relationship between the three different spider silks are investigated. Successful spectroscopic knowledge of the internal protein structure and optical properties of the spider silks could permit industrial production of silk-based fibres with unique properties under benign conditions.

1. Introduction

Current research in the spider silks involves its potential use as an incredibly strong and versatile material. So far, naturally occurring materials like spider silks are able to meet these demands with a specific strength that is five times that of steel or two times that of Kevlar [1–4]. As biomaterials, spider silks have far-reaching biomedical applications [5,6]. It can be processed into films and scaffolds to perk up tissue regeneration in skin, nerve, bone, and cartilage or to repair ruptured connective tissues such as tendons and ligaments [6–10]. The interests in the spider silks are mainly due to a combination of its remarkable mechanical properties. Spiders employ silk for a variety of different purposes such as web spinning and cocoon construction. It also helps to deposit sperm and is composed of proteins, which are large molecules made up of repeating amino acids that perform many functions in cells and form many biological materials. All spiders produce variety of silks, but the orb-web spider produces seven different silks by using different silk glands. However, two are the nearly universal usage. There are the major ampullate silks produced from the major ampullate gland, also encode the dragline silk consists of a spiral of elastic sticky silk suspended upon a framework of stiff, dry radii and the viscid silk produced by the flagelliform gland, which is used to form the catching spirals of the orb-web. The other spider silks of different glands are engaged as wraps for preys and eggs [11,12]. Some social spiders have additional spinning organ called cribellum. Spiders that possess a cribellum are called cribellate. Cribellate gland produced the highly sticky silk, especially the best fine one which is used to help entangle prey [13]. However, due to the low production rate of silk by the spiders it is not possible for different application field. The high production rate should be possible by enhance the mechanical property of spider silk. The method of UV crosslinking is also well-known in biomedical engineering for enhancing the mechanical properties of biomaterials [14]. In effect of exposing UV radiation on spider silks, the electrons in the material excited and will alter the kinetics of chemical reactions and it promotes the process of enhanced mechanical property [15,16]. If the particular band gap of the biomaterial is known, this should be very easy to excite the electrons from the valence band to conduction band.

For this study, spiders belonging to three different families such as *Stegodyphus sarasinorum* Karsch 1892 (Eresidae), *Argiope pulchella*

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(Thorell1881) (Araneidae) and *Nephila pilipes* (Fabricius 1793) (Nephilidae) were chosen [17–19]. *S. sarasinorum* which is a social spider, spin sheet webs using silks from the cribellate gland. *A. pulchella* and *N. pilipes* are orb-web spiders spin webs habitually by dragline and viscid silk.

The structure-property relationship of the spider silks which may be attributed to the presence of ordered (i.e., crystalline) and disordered (i.e., amorphous) phases within the proteins in the fibrils [20]. Spider silks consist of pleated beta-sheets of alanine-rich motifs, which form the crystalline regions assembled by weak inter-strand H-bonds [20]. The predominant secondary structure of the amorphous glycine-rich matrix consists primarily of random coils and helices [20].

The crystalline and amorphous nature of the cribellate silk of *S. sarasinorum* and the combined dragline and viscid silk of *A. pulchella* and *N. pilipes* spider silks were confirmed by using XRD analysis. The amide I, amide II and amide III band structure of the obtained spider silks were analyzed by using FTIR analysis. From the optical absorption spectra, the optical properties such as optical band gap (E_g), refractive index (n), extinction coefficient (k), optical conductivity (σ) and dielectric constant (ε) of the three different spider silks were reported for the first time. The fluorescent property of the spider proteins were measured by fluorescence spectroscopy analysis. Following the utilization of complimentary experimental methods, we may obtain the valuable information about the structural and optical properties of spider silks.

2. Experimental

The experimental spiders namely *Stegodyphus sarasinorum* (Eresidae), *Argiope pulchella* (Araneidae) and *Nephila pilipes* (Nephilidae) were collected from the Kodaikanal Hills, in the Western Ghats, Dindigul District, Tamilnadu, India. It lies on 10°23′81″N, 77°48′92″E with an elevation of 2133 MSL. The collected live specimens were acclimated to laboratory conditions in separate containers (27 ± 2 °C; RH 75 ± 5%) for a week and they were fed with *Musca domestica* (Diptera) and *Nilaparvata lugens* (Hemiptera). The silk samples of these spiders were collected individually by swiping a spatula in the spider holding container there by wrapping 1–2 m length of thread spin inside the spider holding box. The silk wound plates were kept for about 10 days at ambient temperature and refrigerated until analysis. The obtained silks were characterized by X-ray diffraction pattern using the XPERT-PRO diffractometer system with Cu Kα radiation ($\lambda = 1.5406$ Å) and the 2θ range was scanned from 10° to 60° continuously with rate of 2° per minute. The FT-IR spectra of spider silks were characterized by FT-IR type IR Affinity Shimadzu spectrometer recorded in the range 400–4000 cm^{-1}. The UV–Vis absorbance spectra of the silks were recorded with a Shimadzu spectrophotometer. The extinction coefficient (k) has been calculated using the relation,

$$K = \alpha\lambda/4\pi, \tag{1}$$

where λ is the wavelength of light. The refractive index of the spider silk has been calculated by knowing the reflectance that can be determined by the formula,

$$R = \frac{\sqrt{1 \pm (1 - exp(-\alpha d) + exp(\alpha d))}}{1 + exp(-\alpha d)}. \tag{2}$$

Using this relation refractive index (n) can be determined by the formula,

$$n = \frac{-(R+1) \pm \sqrt{-3R^2 + 10R - 3}}{2(R-1)}. \tag{3}$$

The optical conductance is obtained using the relation,

$$\sigma = \alpha n c \varepsilon_o = \alpha n c/4\pi, \tag{4}$$

where σ is the optical conductance, c is the velocity of the radiation in the space, n is the refractive index and α is the absorption coefficient. The dielectric constant can be obtained theoretically by the relation,

$$\varepsilon_r = n^2 - k^2 \text{ and } \varepsilon_i = 2nk, \tag{5}$$

where ε_r is the real part of the dielectric constant, ε_i is the imaginary part of the dielectric constant, n is the refractive index of the material and k is the extinction coefficient. Fluorescence spectra of the spider webs were recorded with a spectrofluorometer (RF-5301 pc).

3. Results and discussion

3.1. Structural studies

Fig. 1 shows the XRD pattern of *S. sarasinorum*, *A. pulchella* and *N. pilipes* spider silks. Many spider silks are known for their crystalline regions due to the formation of pleated beta-sheets. In addition to crystalline regions, however silk proteins contain extensive amorphous regions [21]. XRD pattern of amorphous polymer network of cribellate silk produced from the sheet web spinning social spider *S. sarasinorum* is shown in Fig. 1 and also the XRD analysis predicts the identical polymer network of combined dragline and viscid silks of orb-web spiders, *A. pulchella* and *N. pilipes* which is shown in Fig. 1. The three spider samples have broad X-ray diffraction peaks at $2\theta = 17.8$–29.8°, 17–31.8° and 18.6–29.6° for *S. sarasinorum*, *A. pulchella* and *N. pilipes* respectively. The primary reason for dominating amorphous nature of spider silks in the case of orb-web spiders are the crystalline-like structures due to the alanine rich motifs while the glycine-rich motifs are able to build the amorphous structures, 3_1-helices, or fold into other structures depending on the other amino acids in the respective motifs [22]. In our case, the XRD analysis predicts that the predominating amorphous network of orb-web spiders, which is for the most part of other amino acid interactions and folded with other structures [17]. The sheet web spinning social spider shows the same amorphous network as like orb-web spiders. XRD analysis also predicts that the spider silks are in hydrophilic nature. In the hydrophilic nature spider silks contain larger side chain amino acids, as well as charged amino acids [23]. They are limiting the growth of beta sheets. So that Poly (Gly-Ala) region can form a structure with lesser interactions results in the degradation of crystalline region with improved amorphous orientation [24,25]. In previous literature study, Sujatha Sampath et al., reported the X-ray diffraction study of nanocrystalline and amorphous structure

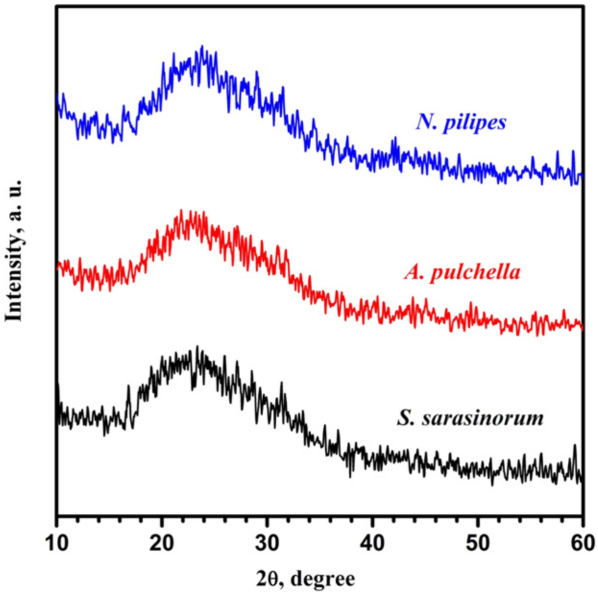

Fig. 1. XRD pattern of *S. sarasinorum*, *N. pilipes*, and *A. pulchella* spider silks.