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Linear and circular dichroism can help us to understand the molecular nature of spider silk

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Abstract
Tougher than steel, synthetic spider silk would have a revolutionary impact in the material sciences with applications ranging from bullet-proof vests to bone implants. A first step towards the development of synthetic spider silk is understanding how the spider produces it. In addition to detailing the morphology and chemistry of the silk production pathway in the spider, it is vital to characterise the molecular protein (Spiroin) constituents of spider silk in both the soluble (in the gland) and insoluble (in the fibre) states. Here we describe the techniques of Circular Dichroism (CD) and Linear Dichroism (LD) spectroscopy for the structural characterisation of spider silk.

Key words: Spider silk, circular dicroism, linear dichroism

INTRODUCTION

The production of silk in the spider depends on the controlled conversion of soluble proteins (members of the Spidroin family) to form insoluble silk fibres (Knight & Vollrath 1999). This process seems to be based on the refolding of the Spidroin proteins (Gosline et al. 1999; Simmons et al. 1996). Spiders take advantage of the low viscosity in the liquid crystalline regime (Magoshi et al. 1985) and extrude an aqueous solution of silk protein to spin molecules into oriented fibres. During the process of spinning, the silk protein solution undergoes an irreversible phase change to a water insoluble solid fibre with high breaking strength and large extensibility (Willcox et al. 1996). The resultant silk fibre is a composite consisting of protein crystals embedded in a protein matrix (Simmons et al. 1996). By varying the relative proportions of crystals to matrix, as well as the length and width of the crystals, the spider can alter the properties of its silk as required (Knight & Vollrath 1999). Previous X-ray diffraction (Becker et al. 1994) and liquid crystalline (Knight & Vollrath 1999; Kerkam et al. 1991) investigations have shown that the silk fibres contain a β-sheet crystalline component and that the fluid secretions exhibit a large-scale helical organisation. The mechanical properties of the final fibre exceed those of many synthetic fibres, which imply a high degree of molecular alignment (Becker et al. 1994). The complexity of the extrusion process suggests that a number of different factors, along the production pathway, contribute to the extraordinary toughness of spider silk. Indeed, silk's extraordinary physical properties (O'Brien et al. 1998) are a consequence of both the composition and molecular architecture of a fibrous protein, Spidroin (Vollrath & Knight 1999; Vollrath et al. 1998).

To understand the formation of silk fibres

in the spider requires an understanding of the Spidroin structure and its conversion on the molecular level. This can help us to understand and predict its function, stability, and intermolecular associations. The structure of Spidroin, like all protein molecules, is organised at different levels. The amino-acid sequence is known as its primary structure. The next level of organisation is called the secondary structure. It is at this level that the three-dimensional structure is first revealed. The secondary structure is defined as the local spatial arrangement of the amino acids. Secondary structural elements form the building blocks that define the total structure of the protein molecule. Several common secondary structures in proteins are the α-helix, β-sheet, and random coil. Some of these can be associated with particular functions whereas others have no specific biological function alone but are part of larger structural and functional assemblies.

CIRCULAR AND LINEAR DICHROISM SPECTROSCOPY

The secondary structure of soluble Spidroin in the gland can be described using Circular Dichroism (CD), while the insoluble Spidroin in the fibre can be analysed using Linear Dichroism (LD) spectroscopy (Rodger & Nordén 1997). CD and LD applications can be largely grouped into (i) monitoring conformational changes; and (ii) determining the secondary structural content. Understanding the secondary structure of a protein can help to clarify the assembly of large molecular organisations of silk fibres. Besides silk, another example of the importance of secondary structure in determining protein assembly and function is the amyloid fibril-forming proteins of pathogenic diseases, such as Creutzfeld-Jacob disease (Jackson et al. 1999). CD and LD are ideally suited to the study not only of the structure of Spidroin, but also its solution-to-fibre conversion.

CD spectroscopy measures the difference in the absorption of left-handed circularly-polarised light versus right-handed circularly-polarised light as a function of wavelength, typically in the visible to ultraviolet region. A preference for one over another only occurs when a symmetric chromophore is located in an asymmetric environment or in the presence of chiral molecules. The absence of regular structure results in a zero CD intensity, while an ordered structure results in a spectrum, which contains both negative and positive signals. CD has a number of advantages over other structural biology techniques. Only a small amount (micrograms) of protein sample is necessary. Indeed, a single silk gland yields enough material for a set of experiments. Also, the protein concentration and buffering can be physiological. Finally, the protein is studied under soluble conditions - which is not possible with x-ray crystallography.

STUDIES OF SPIDER SILK

Already work has been done to begin to characterise the Spidroin conversion in the major ampullate gland of *Nephila edulis* using CD. The gland consists of a sac or ampulla with an apical 'tail', which produces some part of the silk feedstock, and a funnel that leads to the duct. The secretory portion of the gland ampulla has two morphologically distinct zones. Upstream in the silk production pathway the A zone secretes material that makes the silk fibre core and downstream the B zone secretes a coating (Vollrath & Knight 1999). These secretions are passed through a 'funnel' into a long duct. The duct is folded back to itself in an elongated 'S' to give three limbs, which progressively narrow to form a hyperbolic die. The raw solute rich in spider silk protein was carefully extracted from the major ampullate glands of *Nephila edulis* spiders kept under identical conditions. Samples were prepared of raw solute and solute gently diluted (≈1:4) in Schartau's Ringer solution pH 7.4 (Schartau & Leidersher 1983). A small quantity (≈10 µl) of sample was loaded into a 0.01 mm light path quartz cell (Hellma 124-QS). The loaded sample was examined by polarised light microscopy to con-

firm that it had not suffered from mechanical shear-induced polymerisation.

The results of this first study show that the soluble Spidroin in the gland undergoes a dramatic structural conversion (Kenney et al. subm.). Material isolated from different parts of the gland (upstream in the A zone and downstream in the B zone) exhibit two completely different CD spectra. Thus, during passage in the gland, Spidroin is converted from one conformational state to another. The suggestion is that the spider takes advantage of these two states to control the production, transportation and conversion of Spidroin in the gland so that Spidrion can be kept in a soluble state at high concentration (≈ 50%) just prior to spinning the insoluble silk fibre. These results illuminate three important aspects of the conversion of the soluble Spidroin: 1) there is a refolding of the protein structure in the gland, 2) the refolding can be induced, and 3) that the converted state is stable and irreversible. Further CD measurements will be performed on silk molecules in solution to determine as a function of location in the gland: (i) whether the proteins are folded, and if so, characterise their secondary structure; (ii) the conformational stability of the proteins under stress; (iii) environmental effects (e.g., pH); and finally, (iv) whether protein-protein interactions alter the conformation of protein. The CD experiments are ongoing.

The LD principle (optical anisotropy) is the same as the CD except that linearly polarised light is used instead of circularly polarised light and one measures differences in the absorption of light linearly polarised parallel and perpendicular to an orientation axis of the fibre. CD is not an optimal technique for studying the insoluble, linearly organised structures of silk fibres. LD, on the other hand, is ideal for this. LD will be used to access: (i) information about how the chromophores are oriented in anisotropic media; and (ii), information about the mobility and binding of particular centres; and finally, (iii) assignment of transition moment directions. The collected data can be used to understand the organisation of oriented structures, such as crystalline domain within the fibre and their effect on the fibre macroscopic properties.

The extremely high brightness and wide spectral range (from near infrared to vacuum ultra violet) synchrotron radiation (SR) has been recognised as a valuable source for CD (Sutherland 1996). SR CD available on the photobiology facility on the ASRID storage ring, ISA, has recently been developed. This is the only CD facility using a synchrotron radiation source (SRCD) in continental Europe, and one of three worldwide. This new SR CD facility allows CD and LD data to be collected with high spatial resolution and low noise background, which is not possible with a conventional commercial machine. Development of CD and LD on the synchrotron is especially interesting in that one can access shorter wavelength (down to 130 nm), and thereby extend classical analysis of spectra in order to obtain more accurate structural features.

PERSPECTIVES

A more complete understanding of spider silk offered by CD and LD, especially on the molecular level, will not only have a strong impact on building new materials but should also have implications in the understanding of the structural changes associated with biological fibre formation in general. This may be particularly interesting in medicine for pathogenic amyloid fibrils such as α-synuclein in Parkinson's disease (Jensen et al. 2000). Amyloid fibrils are insoluble aggregates that result from the self-assembly of partially unfolded proteins (Cohen & Skinner 1990; Zhang & Rich 1997). We conclude that the application of CD and LD techniques to silk and other spider products (e.g., venom) is expected to provide us with a better understanding, and perhaps leading to successful mimicking, of the self-assembly of silk and other biological products found in nature.

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