

Mapping Protein Orientation in Spider Silk by STXM – The Effect of Water

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Introduction

Spider silk is among nature's most highly engineered structural materials, in some cases achieving combinations of strength and toughness that are still unmatched by high-performance synthetic fibers. Spiders produce several different types of silk with a specific range of mechanical properties that are used for specific or multiple functions. Dragline silk is a remarkable high toughness fiber that combines both stiffness and extensibility. Such properties are due to the peculiar

block copolymer structure of dragline silk that is composed of alternating alanine-rich hard segments containing β -sheets and glycine-rich soft segments. The alignment of the β -sheets within the fiber is an important factor responsible for the tensile strength of spider dragline silk. To establish the fundamental structure-property relationships in spider silk, it is necessary to characterize the microstructure of silk fibers and to map quantitatively the orientation of the β -sheets.

We have recently used scanning transmission X-ray microscopy (STXM) at the Advanced Light Source to show that it is a valuable technique to characterize the spatial distributions of molecular orientation in silk monofilaments. This technique was used for the first time to quantitatively map the level of orientation of the carbonyl bonds of the polypeptide chains at high spatial resolution (smaller than 50 nm) in *Bombyx mori* cocoon silk [1] and *Nephila clavipes* (*N. clavipes*) dragline silk [2]. To study the orientation, it is necessary to rotate either the sample or the E-vector to probe the linear dichroism of the $C\ 1s \rightarrow \pi^*$ (amide) transition. On the 5.3.2 beamline of ALS the sample was rotated using an *in situ* azimuthal rotation system that we have developed [3]. Our results have shown that dragline silk presents a very fine microstructure in which small highly oriented regions that are bigger than individual β -sheet crystallites are dispersed in a dominant, moderately oriented matrix with several small unoriented domains.

All STXM experiments that we have performed so far on silk were done on thin microtomed sections in the dry state. However, it is well known that water is a good plasticizer for silk and that the mechanical properties of silk fibers depend strongly on their degree of hydration. For example, dragline

silk shows a dramatic and reversible water uptake yielding an important fiber shrinkage called supercontraction. We have thus recently launched a project at the CLS on the effect of water on the protein orientation in dragline silk. One major advantage of the CLS EPU-based STXM is that the E-vector of the X-ray beam is rotated instead of the sample, thus resulting in more rapid measurements and more accurate mapping since there is no need to align images.

Methods

Microtomed samples of *N. clavipes* dragline silk fibers reeled at 0.5 cm/s were prepared as described previously [2] except that the samples were kept at high relative humidity (at least 65%RH) from the spinning of the fibers under controlled conditions in the spider farm at Université Laval, through transport and microtoming at McMaster University, and transport to the CLS. The SM beamline 10ID-1 was used for STXM measurements and the samples were mounted in liquid cells equipped with Si_3N_4 windows [4]. The same sample was studied in the dry state (STXM tank filled with helium) and in the wet state after adding liquid water and sealing the cell. During the measurements, the sample orientation was fixed and maps were recorded with the E-vector of the X-ray beam aligned either parallel or perpendicular the fiber axis. Dichroic ratio and order parameter $\langle P_2 \rangle$ maps were then calculated from absorbance maps as described elsewhere [1-2].

Results

Figure 1 shows the $\langle P_2 \rangle$ orientation maps of the carbonyl groups for a *N. clavipes* fiber in the dry and wet states. $\langle P_2 \rangle$ is equal to 1 and -0.5 for perfect parallel and perpendicular orientation, respectively, and to 0 for random orientation. As can be seen, the carbonyl groups of the silk proteins are preferentially oriented perpendicular to the fiber axis indicating that the peptide chains of the β -sheets are mostly aligned along the fiber axis. The dry fiber presents a very fine homogeneous microstructure of moderately oriented domains. The addition of water to the fiber drastically affects the microstructure of the fiber. It is still homogeneous throughout the sample, but with a much coarser texture and a broader range of $\langle P_2 \rangle$ values. This effect is even more clearly observed by comparing the distribution of $\langle P_2 \rangle$ values for the fiber in the dry and wet states (Figure 2). Both distributions display a Gaussian shape and are centered at a $\langle P_2 \rangle$ value of about -0.1. However, the distribution is much broader for the fiber in the wet state

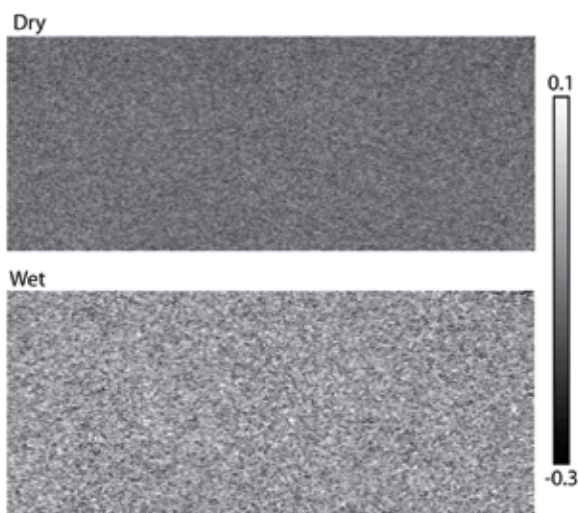


Figure 1: $3 \times 7 \mu\text{m}^2$ maps of the order parameter $\langle P_2 \rangle$ of *N. clavipes* dragline silk in the dry and wet states.

compared to that observed for the dry state. These results show that water induces a major reorganization of the protein chains within the fiber, with some carbonyl groups displaying higher and some lower orientation than in the dry fiber.

As mentioned above, water has a major effect on dragline silk. When unrestrained dragline silk is immersed in water, it shrinks by approximately 40–50%, and its mechanical properties change markedly. The initial stiffness drops by three orders of magnitude, and the material becomes rubber-like [5]. This effect has been associated with the decrease of

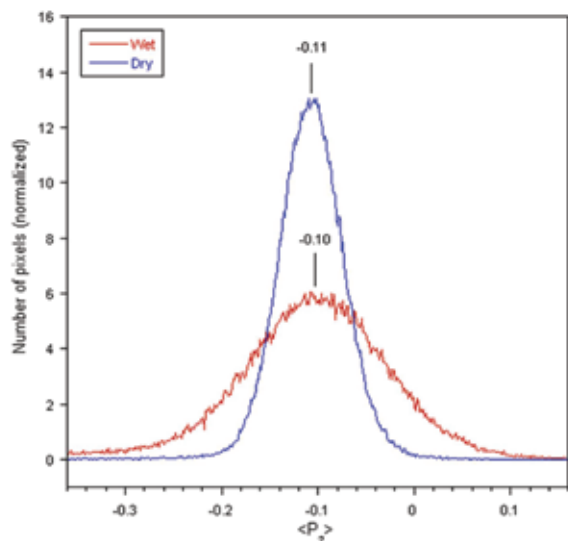


Figure 2: $\langle P_2 \rangle$ distribution (# of pixels with a given $\langle P_2 \rangle$ value) for the orientation maps shown in Figure 1.

interchain interaction in the amorphous matrix in the presence of water, leading to a loss of rigidity. Recent results obtained in our laboratory by polarized Raman spectromicroscopy on unrestrained *N. clavipes* dragline silk supercontracted at about 30% show that supercontraction does not alter significantly the average orientation of the silk proteins but changes slightly their conformation. The present STXM results show that even for a

thin section of dragline embedded in a polymer matrix, water induces effects similar to those resulting from supercontraction. As observed by Raman spectroscopy, hydration does not change the average orientation of the carbonyl groups. However, STXM reveals that water strongly affects the distribution of orientation of the peptide chains. Interestingly, some carbonyl groups become more oriented in the presence of water, suggesting that changes due to silk plasticization by water do not only occur in the amorphous matrix but also in the crystalline domains.

Summary

These preliminary results show that STXM is a unique and valuable technique to follow the effect of water on the spider silk microstructure and the phenomenon of supercontraction. For the first time, it is shown that water broadens considerably the distribution of orientation of the carbonyl groups.

To better control the degree of hydration, a cell with active humidity control is currently under construction. This cell will be used to study the effect of relative humidity as well as supercontraction on the microstructure of silk. The results obtained will be correlated with mechanical properties that are measured at Université Laval on a specially designed fiber stretcher that allows also the simultaneous measurement of polarized Raman spectra. These results should provide new insights into structure-properties relationships in silk samples.

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