Biomimetic spinning of recombinant silk proteins

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ABSTRACT

In the past, we have successfully designed and produced a variety of engineered spider silk-like proteins (eADF3 and eADF4) based upon the primary sequence of the natural dragline proteins ADF3 and ADF4 from the spider *Araneus diadematus* [1]. Genetically engineered spider silk proteins can be modified at the molecular level to optimize the biochemical and mechanical properties of the final product. Although engineered spider silk proteins can be processed into fibers using different spinning methods, our group is interested in the technical realization of a biomimetic approach. Here, we present an overview over our biomimetic fiber production process.

INTRODUCTION

Spider silk has been widely used as a material, long before it came in focus of scientists, for wound dressing (in Ancient Greece), for fishing (Australasia) and later on, for military purposes, e.g. the construction of crosshairs [2, 3]. The variety of applications of spider silk is based on its mechanical stability, biocompatibility, smoothness and thinness in comparison to other available materials [2]. Female orb weaving spiders produce up to 5 different silks with different properties to construct their orb webs [4, 5]. The frame and radii of an orb-web are constructed from dragline silk, whose main constituents are mainly two Major Ampullate Spidroins (MAS). Among all types of silk, dragline silk shows the greatest toughness [2], being five times tougher than steel (on a weight basis) and even three times tougher than man-made synthetic fibers such as Kevlar 49™ [6-8].

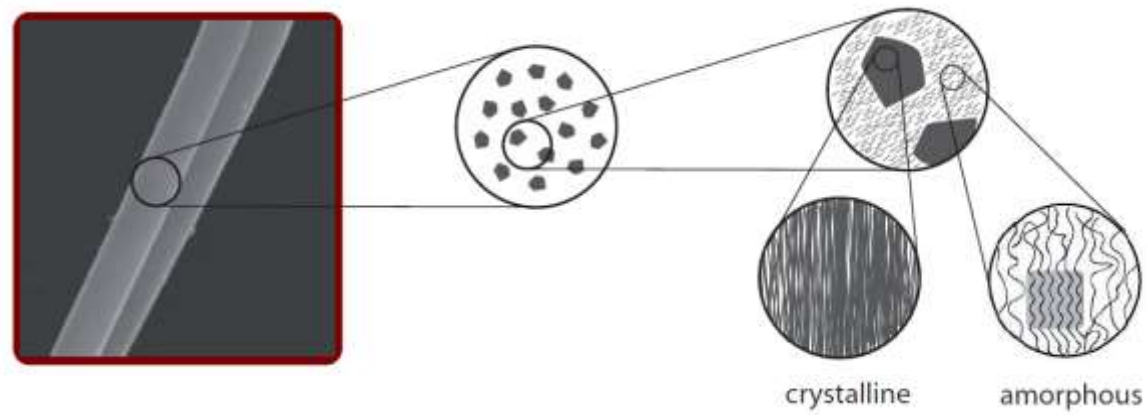

Figure 1. Scheme showing the crystalline and amorphous segments in a dragline silk thread

The MAS are mainly composed of repetitive motifs that are responsible for the formation of amorphous and crystalline regions within the fibers (see Figure 1), which define the fiber's

mechanical properties. During fiber formation, the proteins self-assemble in a complex process involving several physico-chemical steps (see below). In order to employ spider silk for technological applications, our group, amongst others, investigates the various processes accounting for the formation of silk fibers.

SPINNING OF RECOMBINANT SPIDER SILK PROTEINS

Production of recombinant spider silk proteins

The aforementioned fascinating properties of spider silk, and thus its high potential for novel technical applications, has aroused increasing interest in the production of large quantities of spider silk proteins. However, the production of spider silk has experienced numerous problems in the past. Unlike silk producing insects, such as the silkworm *Bombyx mori,* spiders are cannibalistic and territorial, strictly limiting farming and, therefore, the availability of their silk [9]. Therefore, recombinant production of spider silk proteins is considered to be a promising alternative, and remarkable efforts have been made recently [2, 6, 10]: one of the main obstacles *en route* to successfully produce recombinant spider silk proteins is the repetitive sequence found in spider silk genes. Polymerase Chain Reaction (PCR), a common method to reliably amplify genes, is not practicable with repeating sequences. Also, the codon usage of spiders provides challenges for recombinant production at least in prokaryotic hosts. To overcome the aforementioned problems, several attempts to produce spider silk proteins using nonconventional hosts [2] or transgenic animals [11] have been made. However, although each offered certain advantages, they were not promising either due to high costs and/or low yields. "Modern" techniques offer alternatives to specifically design spider silk-like genes [10]. Among others, our group developed a cloning system that allows the engineering of artificial spider silk genes by seamlessly joining solid-phase synthesized oligonucleotides [1]. This method has been described in detail in recent publications and reviews from our group [1, 10, 12]. Based on this new cloning technique, we were able to recombinantly produce a variety of spider silk-like proteins in *E. coli*, based on sequences of the dragline silk proteins of *Araneus diadematus* [1,10, 12].

Step-by-step analysis of spider silk assembly

In order to establish an efficient biomimetic spinning process for spider silk proteins, it is crucial to understand the critical steps involved in the natural process [2]. It is well established that in the natural spinning process a highly concentrated (up to 50% w/v) spinning dope is processed through a spinning duct, where ion exchange and acidification induce a liquid-solid phase transition. In addition to these chemical processes, shear and extensional forces in the duct lead to solidification and fiber formation. Finally, water removal, taking place in the distal part of the duct, is assumed to result in a semi-solid thread exiting the spigot through mechanical drawing, e.g. exerted by the spider's hind legs or by gravity, and by evaporation of residual water in air.

It is important to note that the natural spinning process, in contrast to most synthetic polymer fiber spinning processes, is a complex combination of extrusion and drawing, and thus mimicking the natural spinning process becomes a very challenging technical task [13, 14]. Moreover, in contrast to common technical spinning procedures, where physical

transformation, spinning, and drawing usually appear in sequential order, the process in a spider is rapid, concerted, and simultaneous [7, 15-17].

In order to gain more insight into the natural spinning process, our group in cooperation with a Physics group (Prof. Bausch, TUM), developed a microfluidic device mainly composed of a laminar mixing and an elongational flow module to mimick the natural spinning process [18]. For our studies, we used two different engineered spider silk proteins eADF3 and eADF4, both based on the sequences of the natural *Araneus diadematus* fibroins ADF3 and ADF4 [1]. Our results indicate that fiber formation for eADF3 or eADF3-eADF4 only occurred upon addition of phosphate with a simultaneous pH change and elongational flow [18]. The addition of phosphate to an aqueous eADF3 or eADF4 solution induced the formation of colloidal aggregates [18, 19].

We proposed a model, analogue to a model by Jin and Kaplan, suggesting that for eADF3, hydrophilic side chains stick out of the colloids leading to further interactions with neighboring colloids. Through shear and elongational forces, these colloidal assemblies are forced into contact finally resulting in the formation of fibers [18]. The resulting eADF3 and eADF3-eADF4 fibers were short batch-type fibers and showed high flexibility. For eADF4, no fiber formation was observed since its irreversible colloidal assembly was too rapid [18]. Although this microfluidic setup proved to be a promising research tool for understanding the sequential order and kinetics of silk fiber assembly, it was not possible to form longer continuous fibers, and thus it is not suitable for application in a large-scale technical process. We currently work towards a large-scale technical spinning process based on the integration of above-mentioned findings allowing the continuous spinning of biomimetic silk fibers (figure 2) [20, 21].

Technical adaptation of the natural spinning process

Several factors during the natural spinning process affect the properties of the silk thread. In order to implement these factors into a technical process, their role in the spinning procedure has to be fully understood. Obtaining a concentrated spinning dope was previously limited, due to the solubility of the recombinant silk protein. However, by inducing a liquid-liquid phase separation (occuring at lower critical solution conditions) we were able to obtain a proteinaceous phase with high concentrations [22]. In nature, the liquid-solid phase transition is induced by ion exchange, pH change, and an internal water removal process, all in combination with spin dope fluid behavior and environmental influences. Our biomimetic spinning process is mimicking the liquid-solid phase transition by using a custom-built artificial spinning duct surrounded by a diffusion unit, in which the crucial ion exchange and pH change processes take place. In combination with a subsequent mechanical drawing mechanism fiber assembly is completed.

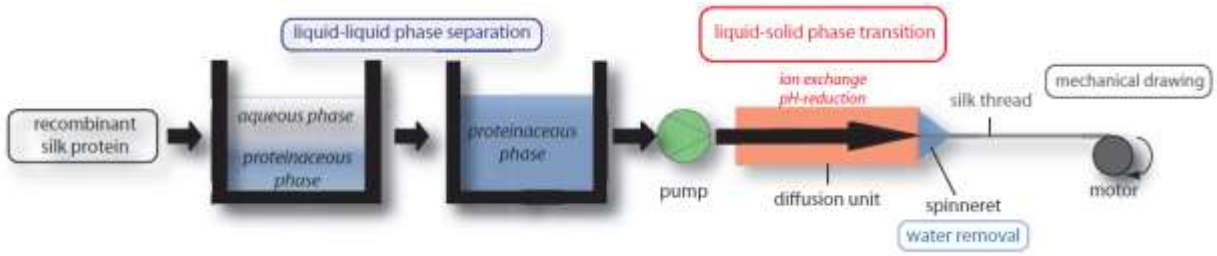

Figure 2. Schematic drawing of a technical biomimetic spinning process

The mechanical properties of these biomimetic fibers will be tested and evaluated in comparison to natural fibers, which we recently investigated in our lab (see figure 3). The obtained data will help us to draw conclusions for the optimization of the biomimetic spinning process.

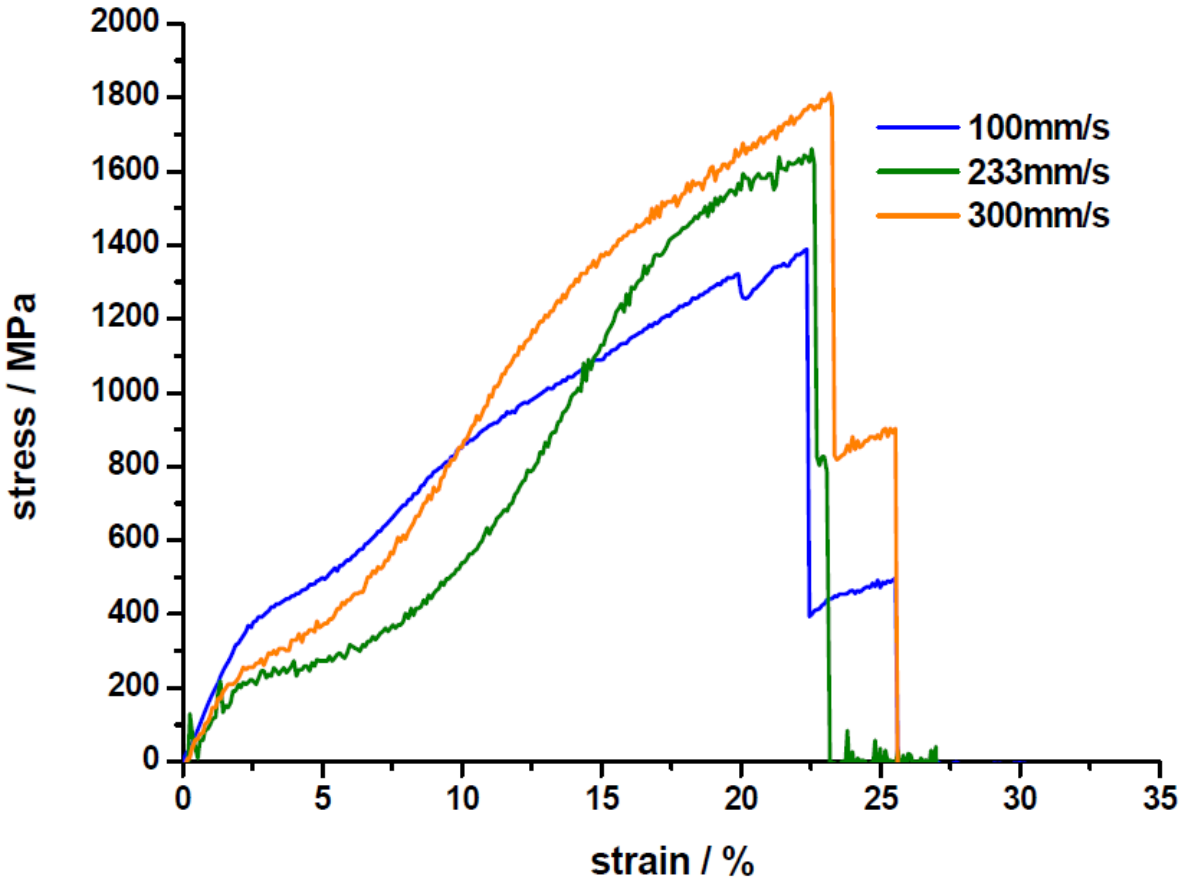

Figure 3. Mechanical data from natural spider silk obtained from *Araneus diadematus* at different silking rates (blue: 100 mm/s, green: 233 mm/s, 300 mm/s)

ACKNOWLEDGMENTS

The work is financially supported by the Bundesministerium für Bildung und Forschung (BMBF) grant number 13N9736.

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