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Mechanisms of the Liquid-to-Solid Transition in Spider Silk Spinning: A Review

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Abstract. Spidroin is stored in the major ampullate gland as a concentrated aqueous dope. The transition occurs in under one second under ambient conditions and yields a fiber of high tensile strength. This review examines four proposed drivers of the transition: the pH gradient, ion exchange, liquid-liquid phase separation (LLPs), and shear and elongational flow. Findings from structural, biochemical, and microfluidic studies are synthesized; no new experimental data are reported. The review synthesizes studies of the N-terminal and C-terminal domains of spidroin, pH changes along the spinning duct, and recent efforts to replicate natural silk spinning using engineered systems. The central question is how these four factors act in combination to convert soluble spidroin into a solid fiber and what this implies for the manufacture of artificial silk. The evidence generally supports a sequential rather than a parallel mechanism: acidification acts first on the terminal domains, ion exchange and dehydration follow, and shear flow completes β-sheet formation. Because each stage depends on the preceding one, single-parameter biomimetic strategies have not reproduced natural silk performance. These findings have implications beyond spider silk itself. A mechanistic understanding of this transition could inform the design of protein-based materials that are processed under mild, aqueous conditions, offering a potential alternative to energy-intensive synthetic fiber manufacturing.

Keywords: Biomimetic spinning, liquid-liquid phase separation, pH-dependent self-assembly, shear-induced alignment, spidroin

1. Introduction

Silk fiber spun by spiders combines high tensile strength with exceptional extensibility, a combination that has proved difficult to reproduce synthetically. Spidroin is stored in the major ampullate gland as a concentrated aqueous dope and is converted into a solid fiber in less than one second. The transition proceeds under ambient temperature and pressure in an aqueous medium, in contrast to the high-energy, organic-solvent routes used for most synthetic fibers. These features account for the sustained interest in the natural spinning process as a model for low-energy fiber manufacture.

The transition has been investigated for several decades. Four candidate mechanisms have been identified as drivers of the transition: a pH gradient along the spinning duct, a shift in ion composition, liquid-liquid phase separation (LLPs), and shear and elongational flow. Each of these

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things may change the shape of the spidroin protein. The literature has not established how the four are coupled, in what order they act, or how faithfully the process can be replicated outside the gland.

This review synthesizes findings from the primary literature and reports no new experimental data. It draws on protein biochemistry, polymer physics, and material engineering. Two questions are addressed: first, how the chemical triggers—pH and ion composition—interact with the physical trigger—shear flow—to convert soluble spidroin into a solid fiber, and second, how closely current biomimetic systems reproduce that conversion. The answers are useful for two reasons. First, it may help people make strong fiber in a way that uses less energy and water. Second, it may help scientists understand how proteins change shape in general.

2. Storage state of spidroin prior to spinning

2.1. Concentration and molecular state of the spidroin dope

Silk production begins in the major ampullate gland, where spidroin is stored as a spinning dope at 30-50 wt% [1]. This concentration is well above that of most naturally occurring protein solutions, and at this point the protein would readily aggregate and solidify prematurely. Within the gland, however, the dope is maintained at a near-neutral pH of approximately 6.9, which sustains solubility [2]. The gland therefore maintains a chemical environment that is finely tuned to suppress premature aggregation; a loss of that control would occlude the duct and halt silk production.

2.2. Functions of the N-terminal and C-terminal domains

Spidroin comprises a long repetitive core flanked by non-repetitive terminal domains. These domains are highly conserved across spider taxa, indicating an important functional role [3]. The N-terminal domain remains monomeric at neutral pH, preventing premature chain association [4, 5]. The C-terminal domain forms a disulfide-linked homodimer that maintains solubility and registers adjacent chains in parallel [6]. Together, the two domains control when and how the protein is converted from a soluble dope into a solid fiber.

3. Chemical regulation during the spinning process

3.1. pH-induced conformational changes

Once the dope leaves the storage sac, it enters the spinning duct, where the pH falls from about 6.9 near the start to about 5.7 along the duct axis [2]. The gradient is generated by ion exchange across the duct epithelium [2]. Acidification triggers a conformational change in the N-terminal domain, which dimerizes and thereby links adjacent spidroin chains [4, 5]. The C-terminal domain responds to the same pH shift, and its rearrangement initiates assembly of the solid fiber [6]. Together the two domains therefore constitute the pH-sensitive relay that controls assembly.

3.2. Ion exchange and dehydration

Concurrently with acidification, the ion composition of the dope changes. Sodium (Na^+) and chloride (Cl^-) concentrations fall while potassium (K^+) and phosphate concentrations rise [7]. This exchange accompanies dehydration of the dope, and the resulting increase in protein concentration drives the system toward its solubility limit [7]. Because both processes are mediated by the same region of the duct, they are spatially and temporally coupled.

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3.3. Liquid-liquid phase separation as an intermediate state

The transition was formerly assumed to proceed directly from dope to solid fiber, but current models identify an intermediate state in which spidroin demixes into micrometer-scale droplets, a process termed liquid-liquid phase separation (LLPs) [8]. Within these droplets the chains are more densely packed and locally ordered, which may permit conformational rearrangement before shear flow completes solidification [8]. Time-resolved imaging has resolved droplet nucleation and growth. Most of this evidence comes from in vitro systems, and it supports, but does not yet establish, a corresponding role in the natural duct.

4. Mechanical assembly and fiber solidification

4.1. Shear-induced alignment of spidroin chains

The duct tapers toward the exit, so the dope is subjected to both shear and elongational flow, which orients the spindroin chains along the flow axis. Polarized-light microscopy of the natural duct has shown that the dope adopts a liquid crystalline phase during flow and that this phase aligns with the flow field prior to full solidification [9].

4.2. β-sheet formation and nanofibril assembly

Once the chains are aligned, the alanine-rich repetitive core adopts a β-sheet secondary structure. Many β-sheets stack into crystalline domains (nanocrystals), which are embedded in a glycine-rich amorphous matrix. This semicrystalline architecture, in which stiff crystalline domains are dispersed in a compliant matrix, accounts for the simultaneous high strength and high extensibility of silk fibers [1].

4.3. Effects of shear conditions on fiber properties

The magnitude and timing of applied shear determine fiber quality. Shear that is too weak yields incomplete chain alignment and low fiber strength, whereas shear that is excessive, or applied before the pH and ion gradients have acted, induces premature solidification. Microfluidic studies have shown that matching both the magnitude and the stage of shear yields fibers approaching the mechanical performance of natural silk [10]. This indicates that shear is a necessary component of the transition rather than a downstream accelerant and that the sequence of triggers, not only their presence, governs the outcome.

5. Biomimetic spinning of artificial spider silk

5.1. Artificial systems that mimic natural spinning conditions

Several approaches have been used to replicate the natural process ex vivo. In wet spinning, a protein solution is extruded through a spinneret into a coagulation bath, which coagulates the protein into a fiber structurally distinct from natural silk [11]. This method does not reproduce the sequencing coupling of pH change, ion exchange, and shear that occurs in the natural duct. Microfluidic platforms offer finer control: channel geometry and composition allow pH, ion concentration, and shear to be varied independently and simultaneously [10].

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5.2. Differences between natural and artificial silk formation

Current biomimetic systems have not closed this gap: artificial silk fiber is still not as strong as natural spider silk [11]. Most devices reproduce only one or two of the four triggers, and few apply them in the right order. The protein itself also differs. Recombinant spidroin produced in heterologous hosts such as bacteria does not experience the gland and duct environment and may therefore fold differently from natural spidroin [11]. It is also frequently shorter than natural spidroin because host expression limits the attainable molecular mass. These differences provide a plausible explanation for the persistent gap in mechanical performance.

5.3. Challenges for industrial-scale production

Scaling the process remains difficult because it requires simultaneous, real-time control of pH, ion concentration, and shear. Such systems are complex to build and run. If that control is achieved, fiber manufacture could rely on aqueous chemistry under mild conditions rather than on the energy-intensive, petroleum-derived routes used for synthetic fibers such as nylon. The associated reduction in solvent use, waste, and energy demand is the principal practical motivation for this line of research.

6. Discussion

Section 5 shows that biomimetic spinning has not yet reproduced the natural process. This suggests something about the natural sequence is still missing from current methods. Future work could focus on applying pH change, ion exchange, and shear force together in the correct order.

The problems described in Chapter 4 point toward some ideas for future work. Machines that control pH, ion levels, and shear force together could solve part of the problem. The natural process inside a spider uses all of these together in a set order.

The order of these steps may matter for a chemical reason. Acid changes the shape of the terminal domains first. Water loss and ion exchange follow, since the protein must lose water before it can separate into droplets. Shear force comes last, because it works only on protein chains that are already partly ordered. Changing this order may stop the protein from reaching the same solid state.

Many natural proteins form solid structures through staged processes. Other proteins may follow a similar pattern. Biomimetic material design may need to copy full sequences of triggers instead of single conditions. Scientists could apply this idea to other materials made from protein.

Fast imaging tools could let researchers watch the protein change as it happens. This could show exactly when shear force should be added during spinning. Spinning machines built from this research could produce fiber closer in strength to natural spider silk over time.

7. Conclusion

This literature reviewed here supports a sequential mechanism. pH change affects the ends of the spidroin protein first. Ion exchange causes water loss and phase separation of the middle part of the protein. Then, shear lines up the protein and makes it solid, full of beta-sheets. These three things answer the question from the introduction. The three stages are coupled rather than independent, since each depends on the state established by the preceding one. This coupling accounts for the limited success of single-parameter biomimetic strategies, such as pH adjustment or shear alone, in reproducing natural silk.

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This paper also has some limits. It uses information from other papers, not new lab data. Most studies about this topic look at one kind of spider silk, called dragline silk, so the ideas here may not be true for other kinds of spider silk. Also, different studies use different numbers for shear force and pH, which makes it hard to compare them directly.

In the future, scientists could keep studying this topic. They could try to build machines that copy the whole process, not just one part of it. They could also try to watch the protein change in real time to see the steps more clearly. Scientists could also try to make this process work on a bigger scale so factories could use it to make large amounts of strong, eco-friendly fiber.

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