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Study Of The Crystalline And Amorphous Regions Of Silk Fibroin

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Abstract: This article presents information on the structure of the structural crystalline and amorphous regions of the fibroin protein in natural silk fiber. The study of the crystalline and amorphous regions in silk fiber provides valuable information on the relationship between molecular structure and macroscopic properties.

Keywords: Silk fibroin, crystalline and amorphous regions, molecular structure, heavy chain, light chain.

Introduction

Silk represents a widely exploited biopolymer. In particular, silk from silkworms has been used in the textile field for over 4000 years due to its excellent mechanical properties, softness, and smoothness. Thanks to centuries of silkworm breeding optimization, SF from silkworm cocoons can be produced easily and in large amounts

Natural Silk fibroin (SF), the primary protein component of natural silk fibers, exhibits remarkable mechanical and biochemical properties such as high tensile strength, elasticity, and biocompatibility [1]. It is widely utilized in biomaterials, tissue engineering, and regenerative medicine. Structurally, silk fibroin consists of two main domains: crystalline β -sheet regions and amorphous random-coil regions [2]. The ratio and organization of these domains determine the overall mechanical behavior, thermal stability, and degradation rate of silk fibroin materials.

Silk fibroin, secreted by *Bombyx mori*, is primarily composed of glycine, alanine, and serine amino acids. The β -sheet crystalline regions are formed by repeating

sequences of (Gly-Ala-Gly-Ala-Gly-Ser)_n, which provide structural rigidity [2][3]. The amorphous regions, by contrast, consist of irregular amino acid arrangements that link the β -sheet blocks and provide flexibility [4]. Silk fibroin is the main component of silk, accounting for about 75% of the total weight of silk. Silk fibroin consists of two leading chains, a heavy (H-) chain (390 kDa) and a light (L-) chain (26 kDa), which are linked by disulfide bonds to form the HL complex. P25 (25 kDa) is a glycoprotein, including ASN linked oligosaccharide chain, which is hydrophobically linked to H-L complex. Three structural protein subsets (heavy chain, light chain, and glycoprotein P25) comprise silk fibroin with a molecular ratio of 6:6:1. Among them, the H- chain of silk fibroin has amphiphilic properties, including hydrophobic and hydrophilic blocks. The hydrophilic region is short and non-repetitive, but the hydrophobic region has repetitive sequences. It can be folded into a β -sheet to generate a crystal structure, so silk fibroin can

be considered a hydrophobic glycoprotein and is insoluble in water [5].

This dual-phase molecular design creates a balance between strength and extensibility: β -sheet domains ensure mechanical resistance, while amorphous regions contribute elasticity and processability. The crystalline and amorphous phases in silk fibroin are strongly influenced by environmental and processing parameters. The crystalline phase (Silk II) corresponds to β -sheet conformations stabilized by hydrogen bonds, while the amorphous phase (Silk I) represents random coil or α -helix structures [1][4].

During spinning and film formation, an amorphous Silk I phase gradually transforms into a crystalline Silk II phase through molecular alignment — this transformation is known as the Silk I \rightarrow Silk II transition [6]. Mechanical stretching, solvent exposure (e.g., methanol, ethanol), and thermal treatment can accelerate this transition.

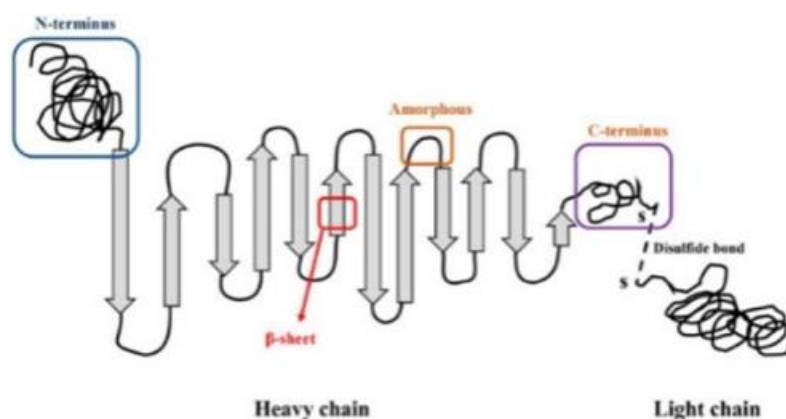


FIGURE 1. Schematic diagram of the silk structure [7].

The primary structure of heavy chain (Hc) is the key for the structural and biological roles of SF, being characterized by a complex aminoacidic composition and patterning. The four most represented amino acids are as follows: glycine (Gly, 46%), alanine (Ala, 30%), serine (Ser, 12%), and tyrosine (Tyr, 5%). The highly repetitive patterns present in Hc can be classified into four principal typologies: (I) GAGAGS, a pattern playing a critical role as a core component of the SF crystalline region; (II) GAGAGVGy–GAGAGY–GAGAGV, which are three sequences rich in aromatic and hydrophobic residues, principally located in the semicrystalline region

of the protein; (III) this pattern is highly similar to (I) but has the presence of an “AAS” β -sheet breaking pattern located at the c-terminal; (IV) these hydrophilic, non-repetitive patterns, which are also called linkers, lack a high-ordered structure, and hence, they may be found in combination with crystallized regions in SF. Patterns such as (IV), in particular, have different aminoacidic compositions and are interspersed through the (I, II, III) patterns. Globally, 12 (I, II, or III) repetitive patterns interspaced by 11 (IV) non-repetitive amorphous regions are present in the primary structure of Hc (Figure 1).

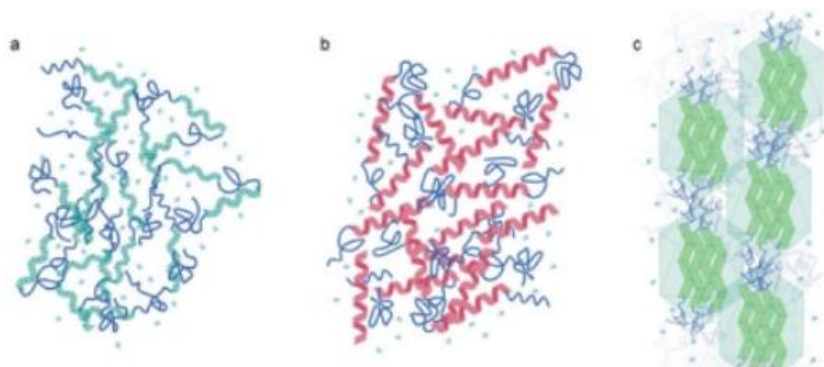


Figure 1. Representation of the patterns of Hc's repetitive or non-repetitive regions

In this picture, (a) Amorphous and unstructured SF conformational states typical of SF aqueous solutions. (b) Silk I form, characterized by the predominance of α -helix structures naturally present in silk glands and reproducible in the laboratory by slow drying, freezing-induced crystallization, and other methods. (c) Silk II form, with a high density of β -sheet crystallites, is produced by silkworms during the spinning process; this SF form can be obtained by several techniques due to the spontaneous tendency of SF to assume this conformation.

METHODS

The first step for SF preparation is known as degumming, consisting of the separation of SR and fibroin to compose raw silk. This process can be carried out with different techniques, leading to differences in process costs and the integrity of the resultant SF. Actually, the main parameter that must be controlled during the degumming process is the structural integrity of the fibroin, which can be controlled by reducing the potential variations in the molecular weight of the biopolymer chains. The structural degradation of the protein can importantly affect the quality of the final product, leading to a decline in the structural and mechanical characteristics of related product forms such as thin films, hydrogels, and porous structures. A canonical degumming method consists of boiling raw silk fiber cocoons in a buffer composed of 0.02 M sodium carbonate (Na_2CO_3) for 30 to 60 min, followed by different washings steps with pure water and the drying of the SF degummed fibers. However, a long-lasting boiling process may lead to variations in the molecular weight of the fibroin; therefore, boiling duration should be monitored to preserve the integrity of the protein. Alternative degumming methods, such as enzymatic degumming or methods making use of concentrated urea or borate solutions, have been studied to optimize

reagent waste and final product integrity. A recently proposed alternative degumming method is based on heating raw silk fibers using microwaves to generate a more efficient and uniform distribution of the heat in the solution and enable better preservation of the structural integrity of the protein. Despite the proposed methods responding to the required preservation of SF integrity, the canonical sodium carbonate technique remains one of the most used methods for SF degumming, and the structural integrity of the obtained SF can be easily checked by fluorescence spectrophotometry. In this respect, less degraded SF samples will maintain a higher tryptophan/tyrosine fluorescence ratio than the highly degraded samples, and this occurrence can be assessed through subjecting diluted degummed samples to excitation at 280 nm, which generates an emission spectrum with a maximum at 307 nm and a shoulder at 330 nm, whereas the intensity of the shoulder peak decreases for increased SF degradation [6]. Depending on the application areas, fibroin is further processed for use in subsequent stages.

Several analytical techniques are commonly employed to investigate the crystalline and amorphous fractions of silk fibroin:

- X-ray diffraction (XRD): used to determine lattice orientation and crystallinity. By analyzing the breadth of the diffraction peaks (especially the equatorial reflections in the pattern), researchers can determine the lateral dimensions of the crystallites, which are ribbon-like filaments in fibroin [2].
- Fourier-transform infrared spectroscopy (FTIR): identifies β -sheet and amorphous domains via characteristic amide I and II peaks [3].
- Raman spectroscopy: detects conformational changes and side-chain vibrations [4].

- Differential scanning calorimetry (DSC): evaluates phase transition behavior and thermal stability [6].

These complementary methods provide comprehensive insights into the structure–property relationship of silk fibroin.

RESULTS AND DISCUSSION

Experimental studies indicate that the crystalline fraction of silk fibroin generally constitutes 40–60% of its structure [2]. Higher crystallinity improves tensile strength, stiffness, and water resistance, whereas a larger amorphous fraction increases flexibility and water absorption [3][4].

The degumming process (removal of sericin) and post-treatment conditions such as annealing and solvent exposure can substantially modify the β -sheet content. Regenerated silk fibroin (RSF) films initially display amorphous conformations, but their crystallinity can be increased by stretching or methanol treatment [1][6].

CONCLUSION

The study of crystalline and amorphous regions in silk fibroin provides valuable insights into the relationship between molecular structure and macroscopic properties. The β -sheet crystalline regions ensure mechanical integrity, while amorphous regions enhance elasticity and processability [1][2]. By precisely controlling the Silk I \rightarrow Silk II phase transition, silk fibroin-based sorbents, biomaterials can be engineered to achieve desired mechanical and degradation characteristics suitable for various biomedical and industrial applications.

Acknowledgement

A review of the literature provides an illustration [6,9,10]:

1. Processing conditions that affect crystallinity include:

Degumming and Regeneration:

The process of removing sericin and regenerating silk fibroin from aqueous solutions can destroy hydrogen bonds and lead to amorphous structures.

Solvents and pH:

The choice of solvents, pH, and salt concentrations during processing significantly affects the destruction or creation of hydrogen bonds, influencing the

transformation between crystalline and amorphous regions.

Mechanical and Thermal Treatments:

Techniques like freezing, heating, and mechanical agitation can induce conformational changes in fibroin, affecting its hydration and promoting either gelation or solidification into crystalline forms.

1. Balance Between Crystalline and Amorphous Regions.

Mechanical Properties:

The ratio of crystalline to amorphous regions dictates mechanical properties:

High Crystallinity: Leads to high tensile strength and stiffness but reduced ductility.

High Amorphous Content: Increases chain mobility, resulting in greater flexibility and ductility

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