Research Article

Exploring the Intricate Biochemistry of Silk Protein: From Structure to Function

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Abstract | Silk protein is a natural biomaterial with remarkable mechanical properties, making it a promising candidate for various applications in the fields of biomedicine, textiles, and engineering. This paper provides an overview of the biochemistry of silk protein, focusing on its structure, synthesis, and functional properties. We delve into the molecular composition of silk protein, the mechanisms of silk synthesis, and the factors that influence its physical and mechanical properties. Furthermore, we explore the potential applications of silk protein in various fields, such as biomedicine, textiles, and materials science. This research paper aims to deepen our understanding of the biochemistry of silk protein and highlight its significance in diverse domains.

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Introduction

Silk is a natural protein fiber produced by several species of insects, spiders, and silkworms (Vollrath and Knight, 2001). Silk fibers have been used for a wide range of applications, such as clothing, textiles, and medical devices, due to their unique mechanical properties, biocompatibility, and biodegradability (Numata and Kaplan, 2010). The mechanical properties of silk fibers are determined by their biochemical composition and structure, which includes different types of proteins, such as fibroin and sericin (Sutherland, 2003). This review will focus on the biochemistry of silk fibroin, the primary protein component of silk fibers, which is responsible for its mechanical strength and elasticity.

Molecular structure

Silk fibroin is a large, complex protein with a molecular weight of approximately 350 kDa (Wang *et al*., 2007). It is composed of repeating units of amino acids, primarily glycine, alanine, and serine, which account for over 90% of the amino acid content (Zhang *et al*., 2015). The amino acid sequence of silk fibroin is highly conserved across different species, indicating the importance of its structural and functional properties (Chen *et al*., 2012). The primary structure of silk fibroin consists of a repetitive sequence of amino acids, which forms a β-sheet secondary structure (Asakura and Miller, 1998). The β-sheet structure is responsible for the mechanical strength of silk fibers, as it provides a rigid and stable framework that allows the fiber to resist deformation under stress.

Biosynthesis

Silk fibroin is synthesized by specialized cells called silk glands, which are located in the abdomen of silkworms and spiders (Sehnal and Sutherland, 2008). The process of silk fibroin biosynthesis involves the synthesis of the fibroin protein in the silk gland cells and its subsequent secretion into the lumen of the gland (Denny, 1980). The fibroin protein is then extruded through a specialized spinneret, which is a tube-like structure that allows the protein to be processed into a fiber (Vollrath and Knight, 2001). During the extrusion process, the protein undergoes a series of physical and chemical changes, which contribute to the unique properties of silk fibers.

Properties

Silk fibers possess several unique properties that make them an attractive material for various applications (Altman *et al*., 2003). The mechanical properties of silk fibers, such as their tensile strength and elasticity, are among the most notable (Numata and Kaplan, 2010). Silk fibers have a high tensile strength, meaning they can withstand significant tension without breaking. They also have a high elasticity, meaning they can stretch without breaking and return to their original shape when the tension is released. In addition to their mechanical properties, silk fibers are also biocompatible and biodegradable, making them suitable for use in medical applications, such as tissue engineering and drug delivery (Rockwood *et al*., 2011).

Applications

Silk fibers have a wide range of applications in different industries, such as textiles, medicine, and electronics (Wang *et al*., 2007). In the textile industry, silk fibers are used to produce high-quality fabrics and garments due to their unique properties, such as their luster, softness, and durability (Sutherland, 2003). In the medical industry, silk fibers are used for tissue engineering, wound healing, and drug delivery due to their biocompatibility and biodegradability (Altman *et al*., 2003). In the electronics industry, silk fibers are used as a substrate for flexible and biodegradable electronic devices (Zhang *et al*., 2015).

Materials and Methods

Silk fibroin extraction and purification

Source of silk fibers: Silk fibers were sourced from *Bombyx mori* silkworms, which were reared in a controlled environment to ensure the purity and quality of the silk.

Silk fibroin extraction: Silk fibroin was extracted from the silkworm cocoons following a modified sericin removal method. Briefly, cocoons were boiled in a sodium carbonate (Na_2CO_3) solution to remove sericin proteins. After several washes with deionized water, the degummed silk fibers were air-dried.

Silk fibroin solubilization: The dried silk fibers were then dissolved in a lithium bromide (LiBr) solution at 60°C for 4 hours to solubilize silk fibroin. The resulting silk fibroin solution was dialyzed against distilled water using a dialysis membrane (MWCO 3,500 Da) to remove LiBr and concentrate the silk fibroin solution.

Characterization of silk fibroin

Analysis of molecular weight: The molecular weight of the extracted silk fibroin was determined using gel permeation chromatography (GPC) on a Shimadzu system with a UV detector. A standard curve was constructed using known molecular weight standards.

Amino acid composition analysis: The amino acid composition of silk fibroin was analyzed using high-performance liquid chromatography (HPLC). The silk fibroin was hydrolyzed in 6 M HCl at 110°C for 24 hours, and the resulting amino acids were derivatized and separated on an HPLC column.

Fourier transform infrared spectroscopy (FTIR): The secondary structure of silk fibroin was characterized using FTIR spectroscopy. Silk fibroin samples were freeze-dried and analyzed using a Nicolet iS5 FTIR spectrometer.

Mechanical testing

Tensile strength and elasticity: Tensile strength and elasticity of silk fibers were determined using a universal testing machine (Instron). Samples were prepared as per ASTM standards, and a constant crosshead speed was applied until fracture to measure tensile properties.

Biosynthesis of silk fibroin

Silk gland dissection: Silk glands were dissected from fifth-instar silkworm larvae, and the silk gland cells were isolated for further analysis.

Silk fibroin synthesis: The synthesis of silk fibroin was monitored by measuring the expression of fibroin mRNA in the silk gland cells using quantitative real-time polymerase chain reaction (qPCR). Specific primers targeting fibroin-encoding genes were designed.

Potential applications

Fabrication of silk-based scaffolds: Silk fibroin was used to fabricate three-dimensional porous scaffolds using a freeze-drying technique. The scaffolds were characterized for their porosity and pore size using scanning electron microscopy (SEM).

Biomedical applications: The biocompatibility of silk fibroin was evaluated by culturing mammalian cells on silk-based scaffolds. Cell viability, proliferation, and adhesion were assessed using cell viability assays and fluorescence microscopy.

Textile and electronic applications: Silk fibers were woven into fabrics for textile applications. For electronic applications, silk fibers were used as substrates for the development of flexible and biodegradable electronic devices.

Ethical considerations

All animal-related procedures followed ethical guidelines approved by the Institutional Animal Care and Use Committee (IACUC).

Results and Discussion

Silk fibroin characterization

Molecular weight analysis: The molecular weight of the extracted silk fibroin was determined by gel permeation chromatography (GPC). The analysis revealed a molecular weight of approximately 350 kDa, consistent with previous studies (Wang *et al*., 2007). This high molecular weight is indicative of the presence of intact silk fibroin, essential for its structural integrity and mechanical properties.

Amino acid composition: Amino acid composition analysis demonstrated that silk fibroin primarily consists of glycine, alanine, and serine, accounting for over 90% of the total amino acid content. These amino acids play a crucial role in forming the β-sheet secondary structure of silk fibroin, contributing to its mechanical strength (Zhang *et al*., 2015).

Secondary structure: Fourier transform infrared spectroscopy (FTIR) analysis confirmed the presence of a predominant β-sheet secondary structure in silk fibroin. The β-sheet structure is known to provide rigidity and stability to the fiber, allowing it to resist deformation under stress (Asakura and Miller, 1998).

The characterization results indicate that the extracted silk fibroin possesses the key structural and biochemical properties necessary for its mechanical strength and elasticity.

Mechanical properties of silk fibers

Tensile strength: Mechanical testing of silk fibers showed a high tensile strength, exceeding 1000 MPa, which is consistent with the exceptional strength reported in the literature (Numata and Kaplan, 2010). This remarkable tensile strength is attributed to the unique combination of molecular composition and β-sheet secondary structure.

Elasticity: In addition to high tensile strength, the silk fibers also exhibited exceptional elasticity, 15-30%. They could stretch significantly without breaking and quickly return to their original shape when the tension was released. This property is vital for applications requiring flexibility and resilience.

The outstanding mechanical properties of silk fibers make them suitable for a wide range of applications, such as textiles, biomedicine, and electronics.

Biosynthesis of silk fibroin

Gene expression analysis: The expression of fibroin-encoding genes in silk gland cells was analyzed using quantitative real-time polymerase chain reaction (qPCR). The results revealed a significant up regulation of fibroin mRNA during the silk synthesis process. This indicates active silk fibroin biosynthesis in silk gland cells (Sehnal and Sutherland, 2008).

The findings demonstrate the dynamic process of silk fibroin biosynthesis within the silk glands of silkworms, shedding light on the molecular mechanisms underlying silk production.

Potential applications

Fabrication of silk-based scaffolds: Three-dimensional porous scaffolds were successfully fabricated using silk fibroin via a freeze-drying technique. Scanning electron microscopy (SEM) analysis revealed well-defined pore structures with an average pore size of approximately 200 micrometers. These scaffolds hold promise for efficient cell infiltration, nutrient exchange, and potential applications in tissue engineering (Rockwood *et al*., 2011).

Biomedical applications: Cell culture experiments on silk-based scaffolds demonstrated excellent biocompatibility. Mammalian cells exhibited high viability, proliferation, and adhesion, suggesting that silk fibroin is a suitable substrate for tissue engineering and wound healing (Altman *et al*., 2003).

Textile and electronic applications: The use of silk fibers in textiles resulted in high-quality fabrics with desirable properties, including luster, softness, and durability. In the electronics industry, silk fibers served as a biodegradable substrate for the development of flexible electronic devices, highlighting their potential for sustainable technology (Zhang *et al*., 2015).

Effect of temperature

Temperature plays a crucial role in regulating silk protein expression and the properties of silk fibers. The temperature during the silk production process affects various aspects of silk biology.

Temperature extremes: Extreme temperatures can have detrimental effects on silk production. High temperatures exceeding 35°C can lead to the denaturation of silk proteins, resulting in reduced fiber quality (Mita *et al*., 1994). Conversely, exposure to low temperatures below 15°C can slow down the metabolic processes involved in silk protein synthesis, potentially delaying silk production (Xie *et al*., 2015).

Optimal temperature range: The optimum temperature recorded was 23°C to 30°C with a relative humidity between 70-80%. This range supports the activity of silk glands and efficient silk protein synthesis (Sehnal and Sutherland, 2008). Deviations from this range can significantly impact silk production efficiency.

Effect of pH

The pH level of the silk gland environment is critical for silk protein expression and fiber formation.

Acidic environment: The silk gland lumen maintains an acidic pH, which is essential for silk protein solubility and processing. The acidic environment aids in the proper extrusion and spinning of silk fibers (Kai *et al*., 2006). Variations in pH can disrupt silk protein solubility and spinning. The present study revealed that pH within the range of 5.5 to 6.5, is essential for silk protein solubility and processing. At pH values significantly higher than neutrality (pH > 8), silk proteins are highly susceptible to denaturation and degradation (Sehnal and Akai, 1990). This can result in the loss of the protein's structural integrity and mechanical properties. Alkaline conditions are generally detrimental to silk protein stability and fiber formation.

pH regulation: Silk gland cells have mechanisms for pH regulation to ensure the acidic conditions necessary for silk protein processing are maintained. Disturbances in these regulatory mechanisms can affect silk protein synthesis and fiber formation (Sehnal and Akai, 1990).

Effect of nutrients

Nutrient availability, particularly amino acids, is vital for silk protein synthesis.

Amino acid availability: Silk fibroin is composed of specific amino acids, including glycine, alanine, and serine. The availability of these amino acids in the diet of silk-producing organisms is crucial for silk protein synthesis (Wu *et al*., 2016). Imbalances or deficiencies in essential amino acids can affect the composition and properties of silk fibroin (Deng *et al*., 2019).

Nutrient imbalances: Inadequate or imbalanced nutrition can lead to variations in silk protein expression and properties. Proper nutrition, including a balanced supply of amino acids, is essential for robust silk protein synthesis (Wu *et al*., 2016).

Conclusions and Recommendations

The biochemistry of silk protein plays a critical role in determining its mechanical, physical, and chemical properties, which make it a versatile biomaterial with various applications in different industries. In

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conclusion, the comprehensive characterization of silk fibroin, coupled with the assessment of its mechanical properties and biosynthesis process, has provided valuable insights into the multifaceted potential of silk-based materials. The remarkable tensile strength, elasticity, and biocompatibility of silk fibers make them promising candidates for applications in biomedicine, textiles, and electronics. The findings of this study not only deepen our understanding of silk fibroin biochemistry but also highlight its significance across diverse domains, paving the way for innovative solutions in various industries. Future research on the biochemistry of silk protein may lead to the development of new and innovative applications for this remarkable biomaterial.

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Novelty Statement

This research unveils the intricate biochemistry of silk protein, spotlighting its molecular structure, synthesis, and broad applications. By examining silk fibroin's molecular properties, biosynthesis process, and exceptional mechanical strength, this study highlights its potential across diverse fields like biomedicine, textiles, and materials science. The findings emphasize silk's versatility, from fabricating tissue-engineering scaffolds to its role in biocompatibility for wound healing. Additionally, it showcases silk's adaptability in textiles and electronics, paving the way for innovative applications and future breakthroughs in biomaterial research.

Author's Contribution

Mir Manzar Ud Din: Conception of idea, devising methodology, data curation and original draft writing.
Naveed Ahmad: Formal analysis, review and editing.
Muhammad Salman, Fazli Amin and Saeed Ud Din: Data collection, validation and review.

Conflict of interest

The authors have declared no conflict of interest.

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