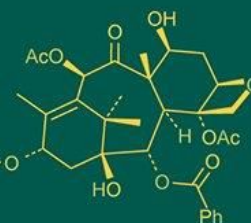
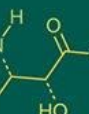
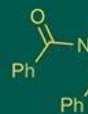


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Exploring the functional variations in fibroin and sericin: Insights into amino acid composition and biomedical applications

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Abstract

The amino acid composition of fibroin and sericin, two primary silk proteins, plays a pivotal role in determining their functional and structural properties. Cocoon fibroin and posterior silk gland fibroin exhibit similar amino acid profiles, with glycine (43.70% and 42.90%, respectively) and alanine (28.80% and 30.00%, respectively) being the most abundant, providing flexibility and structural stability. In contrast, sericin has a distinct composition, with glycine content significantly lower (10.00%), while serine is notably higher (33.40%). This study reveals that while both fibroins share similar amino acid distributions, sericin's high serine concentration enhances its adhesive properties. Other amino acids, such as tyrosine, valine, aspartic acid, and glutamic acid, were also found to vary across the proteins, indicating their role in the unique functionalities of each. The results underline the specialized roles these proteins play, with fibroin contributing primarily to structural integrity and sericin to adhesion. Understanding these variations provides valuable insights for the development of silk-based biomaterials with tailored properties for various industrial and biomedical applications.

Keywords: Fibroin, sericin, cocoon

Introduction

Silk proteins, primarily fibroin and sericin, are natural polymers that exhibit remarkable properties, making them highly versatile for a wide range of applications, particularly in biomaterials, textiles, and biomedical engineering. Fibroin is the principal structural protein in silk, providing the strength and flexibility essential for various applications, while sericin serves as a binding agent that helps hold the silk fibers together, enhancing its functionality. These proteins are composed of distinct amino acid compositions, which significantly influence their mechanical and biological properties. Understanding these differences is crucial for optimising their use across multiple sectors.

Fibroin, which exists in both cocoon and posterior silk gland forms, is predominantly composed of glycine and alanine, amino acids that contribute to the fibrous structure and tensile strength of silk. This composition allows fibroin to exhibit superior mechanical properties, which are beneficial for applications that require durability and structural integrity. In contrast, sericin, which has a higher concentration of serine, possesses hydrophilic and adhesive properties. These characteristics make sericin an essential component for the stability and moisture retention of silk fibers (Sinha *et al.*, 2022) [8]. These differences in amino acid profiles highlight the complementary nature of fibroin and sericin, which together enable silk proteins to serve a wide variety of functions.

Due to their unique mechanical properties, both fibroin and sericin are increasingly used in a variety of applications, ranging from advanced biomedical devices to textiles and sustainable materials. Fibroin's tensile strength and biocompatibility make it ideal for tissue engineering, wound healing, and drug delivery systems. Meanwhile, sericin's moisture-retaining and bioactive properties have proven beneficial in skincare, wound care, and other medical fields (Saad *et al.*, 2023) [6]. Furthermore, the versatility of these proteins extends to non-medical applications such as cosmetics, food packaging, and environmentally friendly industrial materials, showcasing their adaptability.

The distinct amino acid compositions of fibroin and sericin, along with their unique physical and biological properties, allow for a wide range of tailored applications in biomaterials. As the demand for sustainable and bio-compatible materials grows, understanding how these proteins' compositions influence their performance will be key to developing innovative solutions. This study, therefore, aims to examine the specific amino acid profiles of cocoon fibroin, posterior silk gland fibroin, and sericin, to better understand how these compositional variations contribute to their respective structural and functional properties. The insights gained from this research will not only inform the optimisation of silk proteins in current applications but also guide their use in emerging technologies, ranging from medical sutures to biodegradable materials.

Materials and Methods

Bombyx mori silkworms were reared under standard laboratory conditions, and silkworm cocoons were harvested at the end of the pupal stage for protein extraction. The silkworms' cocoons were degummed in a 0.02 M sodium carbonate solution by boiling them at 100°C for 30 minutes, effectively removing sericin. The fibroin was then manually separated from the degummed fibers, washed thoroughly with distilled water, and air-dried. To extract pure fibroin, the fibers were solubilized in a 9.3 M lithium bromide (LiBr) solution at 60°C for 4 hours with continuous stirring. After solubilization, the fibroin solution was dialyzed for 48 hours using a dialysis membrane (MWCO 3 kDa) to remove any residual LiBr. Sericin, the adhesive protein, was extracted from the remaining silk fibers by treating them with 0.02 M sodium carbonate at 100°C for 1 hour. After centrifugation at 10,000 rpm for 15 minutes, the supernatant containing sericin was collected, concentrated using a rotary evaporator, and dialyzed against deionized water for 48 hours.

For amino acid profiling, both fibroin and sericin samples were hydrolyzed in sealed tubes with 6 M hydrochloric acid (HCl) at 110°C for 24 hours. After hydrolysis, the resulting mixture was filtered and evaporated to dryness, with the residue resuspended in 0.02 M HCl for analysis. The amino acid composition of the proteins was determined using High-Performance Liquid Chromatography (HPLC) with a C18 column. The mobile phase consisted of a gradient mixture of methanol and acetonitrile, and the elution was monitored by UV detection at 570 nm after a ninhydrin reaction. Standard amino acid mixtures were used for calibration, and the amino acid profiles of fibroin and sericin were subsequently quantified.

To characterize the purity and molecular weight of the proteins, SDS-PAGE was employed. Equal quantities of the fibroin and sericin samples (10 µg) were loaded onto a 12% polyacrylamide gel, and electrophoresis was performed at 150 V for 2 hours. The gel was then stained with Coomassie Brilliant Blue G-250 and destained to visualize the protein bands. The molecular weights of the proteins were estimated by comparison to a protein ladder. Protein concentration was determined spectrophotometrically at 280 nm using an absorbance reading.

Results and Discussion

The amino acid composition of silk proteins, particularly fibroin and sericin, reveals significant differences that

influence their structural and functional properties (Table 1). The most abundant amino acid in cocoon fibroin was glycine, accounting for 43.70% of the total amino acids, followed by alanine (28.80%) and serine (11.90%). Smaller proportions of tyrosine (5.10%), valine (2.20%), and aspartic acid (1.30%) were also present. Other amino acids, such as glutamic acid (1.00%), threonine (0.90%), and phenylalanine (0.61%), were found in lower concentrations. Methionine was not detected in cocoon fibroin, making it the least abundant amino acid. The composition of cocoon fibroin is predominantly structural, with glycine and alanine providing the key building blocks for its fibrous structure, while serine plays a role in stability and functionality (Kim *et al.*, 2023) ^[2]. The amino acid profile of posterior silk gland fibroin was similar to that of cocoon fibroin, with glycine (42.90%) and alanine (30.00%) as the most prevalent amino acids. However, the concentration of serine (12.20%) was slightly higher compared to cocoon fibroin, which aligns with the findings of Kim *et al.* (2023) ^[2]. Other amino acids, including tyrosine (4.80%), valine (2.50%), and aspartic acid (1.90%), were present at lower concentrations than glycine and alanine, indicating a fine-tuned balance that allows the silk gland fibroin to maintain its structural integrity. Sericin exhibited a distinct amino acid composition when compared to the fibroin fractions. Glycine content was significantly lower in sericin (10.00%), while alanine (16.90%) showed a higher concentration than in the fibroin proteins. Serine (33.40%) was present in notably high amounts, particularly in the smaller subunit of sericin, highlighting its key role in the adhesive and hydrophilic properties of sericin. This higher serine content in sericin aligns with previous studies (Mondal *et al.*, 2007; Zhou *et al.*, 2001) ^[4, 17], which suggest that sericin's function is primarily related to adhesion, water retention, and protective properties, making it crucial for the formation and integrity of the silk fiber. Additionally, aspartic acid (15.40%) and glutamic acid (8.40%) were more abundant in sericin than in the fibroin fractions, further supporting the hypothesis that sericin plays an active role in various biological processes. The smaller subunit of sericin contained a notably higher concentration of serine (33.40%), as well as significant amounts of aspartic acid (16.70%) and glutamic acid (4.40%). This subunit exhibited a lower presence of glycine (13.50%) and alanine (6.00%) compared to the other subunits, indicating a divergence in structural and functional properties. This suggests that different subunits of sericin may have specialized functions, with the smaller subunit potentially contributing more to the protein's adhesive and hydrophilic properties, while other subunits might be involved in structural or protective functions within the silk structure. The findings presented here align with existing literature that emphasizes the structural and functional differentiation between fibroin and sericin. Fibroin, with its predominance of glycine and alanine, is primarily structural, providing strength and stability to the silk fiber. On the other hand, sericin, with its higher serine content, appears to play a critical role in the adhesive properties and hydrophilicity of silk, making it a versatile protein for various applications, particularly in biomedical fields. Overall, glycine, alanine, and serine were the dominant amino acids in the silk proteins, with glycine being the most abundant in the fibroin fractions and serine being more prevalent in the sericin fraction. The amino acid composition highlights the structural differences between

fibroin and sericin, with fibroin contributing to the strength and durability of silk, while sericin's higher serine and

acidic amino acid content supports its role in adhesion and protection.

Table 1: Amino acid composition of silk proteins of *Bombyx mori*

Amino acid	Cocoon fibroin	Posterior silk gland fibroin			Whole sericin
		Whole	Larger sub unit	Smaller sub unit	
Glycine	43.70	42.90	49.40	10.00	13.50
Alanine	28.80	30.00	29.80	16.90	6.00
Serine	11.90	12.20	11.30	7.90	33.40
Tyrosine	5.10	4.80	4.60	3.40	2.60
Valine	2.20	2.50	2.00	7.40	2.80
Aspartic acid	1.30	1.90	0.65	15.40	16.70
Glutamic acid	1.00	1.40	0.70	8.40	4.40
Threonine	0.90	0.92	0.45	2.80	0.53
Phenyl alanine	0.61	0.67	0.39	2.70	0.53
Methionine	-	-	-	0.37	0.04
Isoleucine	0.71	0.61	0.14	7.3	0.72
Leucine	0.51	0.55	0.09	7.20	1.10
Proline	0.31	0.45	0.31	3.00	0.68
Arginine	0.46	0.51	0.18	3.80	3.10
Histidine	0.16	0.19	0.09	1.60	3.30
Lysine	0.31	0.38	0.06	1.50	0.15
Cysteine	-	-	-	1.60	-

The amino acid composition of cocoon fibroin, posterior silk gland fibroin, and whole sericin, including the larger and smaller subunits of sericin, is summarised in the table 2. The most abundant amino acid in cocoon fibroin was glycine, accounting for 43.70% of the total amino acids, followed by alanine (28.80%) and serine (11.90%). Smaller proportions of tyrosine (5.10%), valine (2.20%), and aspartic acid (1.30%) were also present. Other amino acids, such as glutamic acid (1.00%), threonine (0.90%), and phenylalanine (0.61%), were found in lower concentrations, with methionine (not detected) being the least abundant. These amino acids are essential for its fibrous, structural characteristics. Glycine, being the most abundant amino acid, contributes significantly to the flexibility and strength of the silk fibers, while alanine supports the formation of β -sheet structures, contributing to the overall stability and tensile strength (Bunthong *et al.*, 2021) [1]. The amino acid profile of posterior silk gland fibroin was similar to that of cocoon fibroin, with glycine (42.90%) and alanine (30.00%) being the most prevalent. However, the concentration of serine (12.20%) was slightly higher compared to cocoon fibroin. Other amino acids such as tyrosine (4.80%), valine (2.50%), and aspartic acid (1.90%) are observed in smaller amounts, maintaining a composition similar to cocoon fibroin, although with slight variations that might influence its physical characteristics (Bunthong *et al.*, 2021) [1]. Sericin demonstrated a distinct amino acid composition compared to the fibroin fractions. Glycine was significantly lower (10.00%) in sericin, with alanine (16.90%) showing a higher concentration than in the fibroin proteins. Serine was present in high amounts (33.40%), particularly in the smaller subunit of sericin, which indicates its key role in the adhesive and hydrophilic properties of sericin. Additionally, aspartic acid (15.40%) and glutamic acid (8.40%) were more abundant in sericin than in the fibroin fractions. The presence of methionine (0.37%) and isoleucine (7.30%) in sericin further suggests the protein's role in various biological processes. The large subunit of sericin contained higher levels of leucine (7.20%) and phenylalanine (2.70%). Additionally, sericin is enriched with aspartic acid (15.40%) and glutamic acid (8.40%), suggesting its involvement in

various biological functions, including interactions with cells or tissues (Bunthong *et al.*, 2021) [1]. These components, in combination with methionine (0.37%) and isoleucine (7.30%), highlight sericin's potential role in biological processes such as cellular adhesion and immune modulation. The smaller subunit of sericin had a notably higher concentration of serine (33.40%), as well as a significant amount of aspartic acid (16.70%) and glutamic acid (4.40%). This subunit had a lower presence of glycine (13.50%) and alanine (6.00%) compared to the other subunits, indicating the divergence in structural and functional properties between the subunits of sericin.

The amino acid compositions of fibroin and sericin, as shown in Table 3, exhibit distinct differences in their ranges, reflecting their diverse biological functions. Glycine concentrations in fibroin range from 12.5 to 40.0 g/100 g, indicating its high abundance, which is essential for the protein's structural properties, enabling tight packing and crystallinity in silk fibers (Mondal *et al.*, 2007; Kirimura& Suzuki, 1962) [4, 3]. In contrast, sericin contains much lower glycine concentrations, ranging from 0.10 to 5.77 g/100 g, which aligns with its adhesive and more hydrophilic nature. Similarly, alanine levels in fibroin range from 18.5 to 25.0 g/100 g, contributing to the stability and rigidity of the fibroin structure, thereby enhancing the mechanical strength of the silk (Kirimura& Suzuki, 1962) [3]. In sericin, alanine concentrations are more variable, ranging from 0.73 to 9.80 g/100 g, reflecting its function as a glue-like protein, providing flexibility and adhesion (Kirimura& Suzuki, 1962) [3]. Leucine levels in fibroin are between 0.70 and 2.5 g/100 g, while sericin shows greater variability (0.40–5.80 g/100 g). This difference may suggest that leucine plays a more dynamic role in sericin's adhesive function. Aspartic acid concentrations are higher in sericin (2.50 to 6.81 g/100 g) compared to fibroin (0.75 to 2.9 g/100 g), reflecting aspartic acid's role in enhancing sericin's solubility and structural properties (Kirimura& Suzuki, 1962) [3]. Both fibroin and sericin contain similar levels of glutamic acid (0.07 to 3.0 g/100 g in fibroin and 0.94 to 3.0 g/100 g in sericin), although sericin has slightly higher concentrations, suggesting a similar role in stability and functionality for

both proteins ("Physicochemical Analysis, Elemental and Amino Acid Composition of Wool and Silk," 2022) [5]. Serine concentrations are significantly higher in sericin (2.56–6.60 g/100 g) compared to fibroin (0.70–1.75 g/100 g), supporting its more hydrophilic and adhesive properties, which are essential for binding silk fibers together. Proline is present in both proteins, with concentrations ranging from 0.07 to 2.50 g/100 g in fibroin and 0.35 to 3.00 g/100 g in sericin. Proline is crucial for maintaining structural integrity in both proteins, aiding their functional roles in silk formation. Tyrosine levels are higher in fibroin (7.80–11.00 g/100 g) than in sericin (1.00–5.25 g/100 g), reflecting its role in stabilizing the fibroin structure, particularly by contributing to the β -sheet configuration, which imparts strength to the silk. Phenylalanine concentrations are similar

in both fibroin (1.00–1.60 g/100 g) and sericin (0.30–2.66 g/100 g), suggesting that it contributes similarly to the structural and functional properties of both proteins. These differences in amino acid composition between fibroin and sericin are indicative of their respective biological roles. Fibroin, with its higher glycine and alanine content, contributes to the structural integrity and strength of silk fibers, while sericin, with its lower glycine and higher aspartic acid content, serves as an adhesive, providing solubility and flexibility. Such distinct amino acid profiles are essential for the physiological and structural functions of these silk proteins, ensuring silk production with desirable properties for various applications (Bunthong *et al.*, 2021) [1].

Table 2: Distribution of Amino acids (gms) of mulberry leaf protein (10kgs) in silkworm body, fibroin and sericin protein

Amino acids	Mulberry leaf protein	Silkworm body protein	Fibroin (1kg)	Sericin (0.25 kg)	Total protein in silkworm body, fibroin and sericin
Glycine	601.00	197.50	380.00	21.52	599.02
Alanine	517.00	274.5	209.00	9.77	443.52
Serine	611.00	40.12	131.00	74.65	257.65
Tyrosine	368.00	78.37	123.00	9.42	210.79
Valine	711.00	56.49	26.00	4.70	87.19
Aspartic acid	1044.00	70.87	14.00	41.67	126.49
Glutamic acid	1134.00	104.25	12.00	26.55	142.80
Threonine	512.00	63.87	9.00	12.90	85.77
Isoleucine	626.00	9.13	15.00	16.87	41.00
Leucine	874.00	95.37	9.00	2.22	106.59
Phenyl alanine	542.00	18.75	9.00	1.72	29.47
Proline	547.00	85.25	4.00	1.90	91.15
methionine	164.00	15.50	10.00	-	15.50
Cysteine	234.00	-	1.00	1.27	2.27
Lysine	746.00	64.50	4.00	14.37	82.87
Histidine	343.00	33.00	4.00	3.47	40.47
Arginine	517.00	80.25	7.00	10.17	97.42
Tryptophan	22.00	5.25	10.00	1.50	16.75

Table 3: Amino acid range in the fibroin and sericin

Amino Acid	Fibroin	Sericin
Glycine	12.5-40.0	0.10-5.77
Alanine	18.5-25.0	0.73-9.80
Leucine	0.70-2.5	0.40-5.80
Aspartic acid	0.75-2.9	2.50-6.81
Glutamic acid	0.07-3.0	0.94-3.0
Serine	0.70-1.75	2.56-6.60
Proline	0.07-2.50	0.35-3.00
Tyrosine	7.80-11.00	1.00-5.25
Phenylalanine	1.00-1.60	0.30-2.66

Conclusion

The amino acid composition of cocoon fibroin, posterior silk gland fibroin, and sericin demonstrates the unique properties and functions of each protein. While fibroin's higher glycine and alanine content contributes to its strength and flexibility, sericin's elevated serine content plays a crucial role in its adhesive and hydrophilic nature. These differences are key to understanding the multifunctionality of silk proteins and their potential in diverse applications. The information from this study can guide the development of advanced silk-based biomaterials, offering tailored solutions for medical, industrial, and environmental applications. Further research into the specific roles of amino acids in these proteins will be essential for refining their use in biomaterial design.

References

- Bunthong C, Siriamornpun S. Changes in amino acid profiles and bioactive compounds of Thai silk cocoons as affected by water extraction. *Molecules*. 2021;26(7):2033.
- Kim YJ, Kim SW, Kim K, Ki CS, Um IC. Structural Characteristics and Properties of Cocoon and Regenerated Silk Fibroin from Different Silkworm Strains. *International Journal of Molecular Sciences*. 2023;24(5):4965. <https://doi.org/10.3390/ijms24054965>
- Kirimura J, Suzuki T. Studies on Amino Acid Composition of Silk Protein by Microbiological Determination. Part III. *Bulletin of the Agricultural Chemical Society of Japan*. 1962;36(3):261-265. https://doi.org/10.1271/NOGEIKAGAKU1924.35.7_64
- Mondal M, Trivedy K, Kumar SN. The silk proteins, sericin and fibroin in silkworm, *Bombyx mori* Linn.,-a review. *Caspian Journal of Environmental Sciences*. 2007;5(2):63-76. <https://www.sid.ir/En/Journal/ViewPaper.aspx?ID=140809>
- Physicochemical Analysis, Elemental and Amino Acid Composition of Wool and Silk. *Journal of Genetic Engineering and Biotechnology Research*. 2022;4(2). <https://doi.org/10.33140/jgebr.04.02.15>

6. Saad MT, El-Samad LM, Gomaa R, Augustyniak M, Hassan M. A comprehensive review of recent advances in silk sericin: Extraction approaches, structure, biochemical characterization, and biomedical applications. *International Journal of Biological Macromolecules*. 2023;126067.
<https://doi.org/10.1016/j.ijbiomac.2023.126067>
7. <https://doi.org/10.1016/b978-0-323-89928-4.00023-7>
8. Sinha A, Parida PP, Siddavaram S, Chakrapani C, Jena KP, Sathyanarayana K. Sericin-a gift of nature: its applications. *Plant Archives*. 2022;22(Spl. Issue (VSOG)):188-195.
<https://doi.org/10.51470/plantarchives.2022.v22.specialissue.036>