

Sericin, a Versatile Protein from Silkworm - Biomedical Applications

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Abstract

The silkworm, Bombyx mori, which feeds solely on the leaves of the mulberry plant, produces mulberry silk. The components of Bombyx mori silk are 60-80 percent fibroin, 15-35 percent sericin and 1-5 percent non-sericin, including wax, pigments, sugars and other impurities. Silk sericin is a natural polymer that encloses and holds together two filaments of fibroin in the silk thread used in the cocoon. In the research and production of medical biomaterials and biomedicines, protein-based silk fibroin has been widely used for two decades. Sericin is often neglected and abandoned in the manufacturing of traditional silk fabrics, silk floss or synthetic silk biomaterials as a by-product or waste. However, sericin is not only a highly useful biological substance, but also has a great deal of biological activity, similar to fibroin. General characteristics of both silk proteins, fibroin and sericin, extracted from Bombyx mori silkworm, and the biological activity and possible use of sericin were addressed in this review. Due to its amino acid make-up and antioxidant properties, sericin has been used in the food and cosmetic industry. The moisturizing power provides guidance for wound healing as a therapeutic agent, protection against ultraviolet radiation, and formulation of creams and shampoos. Antioxidant activity associated with low sericin digestibility, which increases application in the medical field, such as antitumor, antimicrobial and anti-inflammatory agent, anticoagulant, works in the health of the colon, enhances constipation and, by improving plasma lipid profile, protects the body from obesity. In addition, the properties of sericin allow its application in tissue engineering and drug delivery as a culture medium and cryopreservation, demonstrating its efficient use as an essential biomaterial.

Keywords: *Bombyx mori*, Sericin, Silk proteins, Fibroin, Biological properties

Introduction

Sericulture is a science that deals with silk production by silkworm rearing. There are four types of natural silk that are known and manufactured in the world commercially. Mulberry silk is the

most significant among them and accounts for as much as 90% of world production, so the word ‘silk’ usually refers to the silk of the mulberry silkworm. The silkworm, *Bombyx mori*, which feeds solely on the leaves of the mulberry plant, comes from mulberry silk. India is the second largest producer of silk in the world, second only to China. In India, sericulture is practiced mainly in tropical regions such as Karnataka, Tamil Nadu, Andhra Pradesh and West Bengal, and to a limited extent in Jammu and Kashmir’s temperate climate. With an annual silk production of about 20,000 metric tons, India’s silk production is around 18 percent of the world’s total raw silk production. Karnataka is the leading producer of mulberry silk, accounting for over 50% of the country’s output. The state of Karnataka is now known as the “Silk Bowl of India” and the rural Mysuru and Bengaluru of Karnataka are also renowned for their silks and are called the “Silk City” as they contribute mainly to the production of silk in India (Bukhari et al. 2019). Kolar, Ramnagara, Bengaluru Rural, Chikkaballapura are the other districts in Karnataka that contribute more silk production besides Mysuru and Bengaluru. The Multivoltine and Bivoltine Seed regions of Karnataka are well known. They meet the demand for parental seed cocoons needed for cross-breed and bivoltine hybrid layers to be manufactured. In the southern part of Karnataka, almost 88 percent of Karnataka’s sericulture is spread.

Components of Cocoon

The silk spinning apparatus, called the silk gland of the silkworm, produces silk. Four separate stages of metamorphosis are included in the silkworm life cycle: egg, larva, pupa or chrysalis, and adult (moths). By converting their indigenous gland liquid silk, Silkworms grow silk cocoons to make the fiber by spinning silk fibers that shelter them during their metamorphism time. Conformational transformation of the silk proteins from a random coil or alpha helical structure via an elongational stress to a solid β -sheet conformation. The two primary constituents of *B. mori*’s raw silk fiber are sericin and fibroin. Sericin is around 20-30 percent, while fibroin is the primary component (70-80 percent) and 0.5 percent mineral salts and 1.5 percent fat and wax are minor components, which are likely to serve as a water repellent for the cocoon (Terada et al., 2002).

Silk Fibroin

Silk fibroin (SF) of *B. mori* contains two polypeptide chains: a heavy (H) of molecular weight (mw) 391,367 kDa chain and a thin (L) of mw 25 kDa chain. Additionally, there is a glycoprotein known as P25 in SF. The assembly of H-chains, L-chains and P25 has a 6:6:1 ratio, which is also known to be a SF protein signature. In alpha-helix and random coil conformation, the SF protein remains in the silk glands of silkworms, which during spinning turn to mechanically solid silk fibers or threads. The stability of silk fibers is due to the transition of the SF protein from random coils to β -sheet conformation at the time of silk cocoon construction (Inoue et al., 2000, Konwarh et al., 2016). Due to the existence of repeated stretches of (GAGAGS)_n and (GAGAGY)_n in the protein sequence of SF, the β -sheet structures are largely established. Silk fiber contains glycine and alanine amino acid hydrophobic repetitive domains, which contribute to more than 50% of total fibroin and thus impart crystallinity to the overall protein structure. The high mechanical strength and structural properties of silk is primarily due to its crystalline property. Differences in the crystalline structures often lead to different physical properties due to different amino acid sequences between different silkworms (Konwarh et al., 2016).

Silk Sericin

With successive sticky layers, Sericin covers the fibroin fibers, forming the cocoon, and accounts for 20% - 30% of the total cocoon mass. Sericin can be graded as sericin A, sericin B, and sericin

C in three fractions, depending on their solubility. Sericin A is insoluble in hot water and is the outermost layer. Sericin B is the middle layer which produces amino acid of sericin A on acid hydrolysis, Sericin C is the innermost layer adjacent to fibroin and is insoluble in hot water and can be extracted from fibroin by hot dilute acid or alkali treatment. Owing to a large number of serine residues in the amino acid series, the protein is thought to be called sericin.

Depending on the extraction and processing methods, the molecular weight of sericin varies from 10 to 400 kDa with complex protein biochemistry (Kumar and Mandal, 2017). The content of amino acids varies according to the silkworm type, and so the protein's structural conformation often varies. For example, the structure of *B. mori* silk sericin (BMSS) has 36.1 percent random coils, predominating in other conformations such as turns (35.1 percent) and helix (28.8 percent) (Kumar and Mandal, 2017).

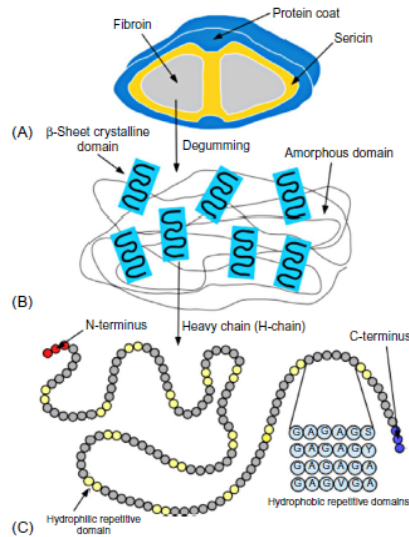


Figure 1: Schematic illustration of silk fibers produced by silkworms: (A) the raw silk fibers consist of two fibers of fibroin kept together with a protein-coated sericin. The fibroin fibers are dissolved in solution after degumming, eliminating sericin; (B) the example of β -sheet crystallite embedded in the amorphous matrix of silk fibroin fibers; and (C) each silk fibroin heavy chain (H-chain) consists of repetitive hydrophobic and hydrophilic domains. (Image from Costa et al., 2018)

Biological Activities and Applications of Sericin

Hypoglycemic Activity

For the treatment of diabetes and related diseases, a scouring soup of silkworm cocoon was prescribed in Chinese medicine in ancient China. A whole silkworm larva's dried powder has a hypoglycemic effect. The increased level of insulin in the blood that significantly promotes insulin secretion is demonstrated by oral administration of sericin to rats. In addition, mice fed a high-fat diet containing a mixture of silk fibroin and sericin had an improved potential for antioxidants and hypoglycemic impact. In T1DM (Type I Diabetes Mellitus) mice, oral administration of sericin extract from hIGF-I-transgenic silkworm cocoons decreased blood glucose levels (Cao and Zhan, 2016).

Improvement of the Physical and Gastrointestinal Function

Silk amino acids have strengthened the endurance of mice and their male reproductive ability. In a rat model of Parkinson's disease, tyrosine-rich silk amino acids have enhanced physical function. Intestinal absorption of zinc, iron, magnesium and calcium was facilitated by the uptake of sericin by rats. Sericin enhanced physiological intestinal functions and demonstrated anti-constipation activity, indicating that buckwheat sericin could have similar effects as dietary fiber, such as anti-constipation (Kunz et al, 2016).

Lowering Blood Pressure and Fat

Sericin peptide intravenous injection (0.1–1000 µg/kg BW) decreased blood pressure and had vasodilatory effects in rats. Silk protein administration with various compositions of fibroin/sericin decreased hyperlipidemia and body fat in mice fed a high-fat diet. They had decreased cholesterol, increased glucose tolerance and elevated serum adiponectin when the rats were fed a high-fat diet containing sericin. Sericin decreased serum absorption of cholesterol and cholesterol in Caco-2 cells in rats (Cao and Zhan, 2016).

Antioxidative and Anti-Tumor Activity

Sericin has been reported to be an antioxidant for a long time. In the intestine, significant quantities of lipid peroxidation are decreased by the antioxidant effects of sericin, thus inhibiting the development of colon tumors. Sericin has been reported to inhibit cloned tumor cell growth and activate the apoptosis factor, leading to cancer cell apoptosis. The undigested colon sericin had a significant antioxidant effect and decreased oxidative stress and colonic tumorigenesis effectively. These findings showed that sericin protein can be beneficial for intestinal health and can be produced as a dietary supplement for colon cancer prevention (Cao and Zhan, 2016).

UV Radiation, Skin Moisturizing and Whitening and Promoting Wound Healing

Due to its anti-UV, whitening, and antioxidant properties, sericin protein was first widely used in cosmetics. Many forms of anti-UV creams, skin and hair care products or baby sericin products have been successfully developed by cosmetic companies in Japan, China and other countries. A sericin-containing anti-UV cream was successfully developed by Hu et al. in 2010, and experiments on rabbits and the human body showed that the anti-UV cream was safe and effective. Sericin is non-toxic to the human body, non-irritating and non-allergenic, making it a natural medication for skin care and a safe anti-UV additive. Moreover, by reducing oxidative stress, it can also protect against acute UVB-induced skin injury in mice.

Drug Delivery System

The powdered sericin particles were crosslinked to sericin-L-asparaginase bioconjugates through a glutaraldehyde biological linker. The bioconjugates of the enzyme are superior in different kinetic analyses to the free enzyme. It was shown by an in vitro hydrolysis test that it had a certain protease resistance. The bioconjugation of sericin-L-asparaginase and sericin insulin uses water-soluble sericin to treat leukemia and diabetes mellitus. Such bioconjugates have slow release characteristics.

Water-insoluble sericin proteins with higher molecular weights (50 to 200 kDa) by using cocoon silk degumming in water under high-temperature high-pressure conditions, and then the powdered sericin particles were cross-linked to sericin-L-asparaginase bioconjugates via a glutaraldehyde biological linker. The enzyme bioconjugates are superior to the free enzyme in various kinetic analyses. An in vitro hydrolysis test showed that it had a certain resistance to proteases. The sericin-

L-asparaginase and sericin insulin bioconjugates using a water-soluble sericin used for treatments of leukemia and diabetes. Such bioconjugates have slow release characteristics. In addition, silk protein and its hydrolyzates have been used to generate iron-binding peptides that in iron-deficient rats can increase bioavailability (Gholipourmalekabadi et al., 2019; Chouhan and Mandal 2019).

Cryopreservation

A main technology for obtaining a continuous source of usable cell lines is cryopreservation. Currently, as a cryoprotectant solution, 10% DMSO mixed with FBS is commonly used. Thus, in recent years, the production of serum-free cryoprotectants has also been a hot subject. In addition to promoting cell growth, the addition of sericin hydrolyzate with an average molecular weight of 30 kDa can be used as an efficient cooling medium for mammalian cells, insect cells, adherent cells and suspension cells (Cao and Zhan, 2016).

3D Scaffolds

Skin 3D scaffolds for tissue engineering structures were developed using fibroblast/keratinocyte cells, and the results showed that autoclaved sericin extracted from cocoons could promote wound healing and the high temperature and high pressure enabled the highest production of collagen by heat-degraded sericin. In addition, genipin crosslinking formed a sericin/poly (N-isopropylacrylamide) hydrogel and was successfully used for cell proliferation and rapid detachment of the thermo-sensitive culture L929. As a biomedical tissue engineering material, a silk sericin-PVA 3D scaffold crosslinked with genipin was also used, particularly in the use of tissue engineered skin substitutes (Cao and Zhan, 2016).

Surface Modifications

Sericin has good biocompatibility and hygroscopic properties and is thus often used in textile materials as a surface modifier. The fabric decreased skin discomfort and the disruption of physiological skin flora resulting from textile contact when the cellulose fiber surface was modified with silk sericin. A composite of sericin/TiO₂ nano coated on a cotton fabric could boost antibacterial effects. Sulfated sericin has an anticoagulant activity similar to silk fibroin, produced through the reaction of chlorine acid. Sericin can enhance the feeling when dentures are used. The aim is to stop, strengthen or relieve different conditions in the dried oral cavity or to improve the denture's stability during use (Cao and Zhan, 2016).

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