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STUDY ON MOLECULAR ARCHITECTURE OF SILK FIBROIN

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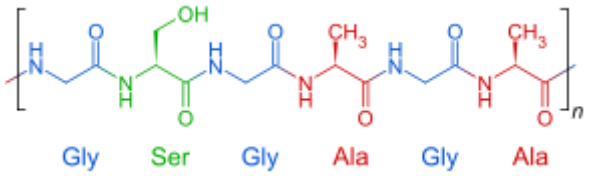
Abstract

The golden silk spun by Indian golden silkmoth *Antheraea assama,* is regarded for its shimmering golden luster, tenacity and value as biomaterial. This report describes the gene coding for golden silk H-fibroin (AaFhc), its expression, and full-length sequence and structurally important motifs discerning the underlying genetic and biochemical factors responsible for its much sought-after properties.

Keywords: silk fibroin, golden silk

INTRODUCTION

Fibroin is an insoluble protein present in silk produced by the larvae of Bombyx mori, other moth genera such as Antheraea, Cricula, Samia and Gonometa, and numerous other insects. Silk in its raw state consists of two main proteins, sericin and fibroin, with a glue-like layer of sericin coating two singular filaments of fibroin called brins.[1][2]



Primary structure of fibroin, (Gly-Ser-Gly-Ala-Gly-Ala)n

The silk worm produces fibroin with three chains, the light, heavy, and the glycoprotein P25. The heavy and light chains are linked by a disulphide bond, and P25 associates with disulphide-linked heavy and light chains by noncovalent interactions. P25 is plays an important role in maintaining integrity of the complex.[3]

The heavy fibroin protein consists of layers of antiparallel beta sheets. Its primary structure mainly consists of the recurrent amino acidsequence (Gly-Ser-Gly-Ala-Gly-Ala)n. The high glycine (and, to a lesser extent, alanine) content allows for tight packing of the sheets, which contributes to silk's rigid structure and tensile strength. A combination of stiffness and toughness make it a material with applications in several areas, including biomedicine and textile manufacture .

Fibroin is known to arrange itself in three structures, called silk I, II, and III. Silk I is the natural form of fibroin, as emitted from the Bombyx mori silk glands. Silk II refers to the arrangement of fibroin molecules in spun silk, which has greater strength and is often used in various commercial applications. Silk

III is a newly discovered structure of fibroin.[4] Silk III is formed principally in solutions of fibroin at an interface (i.e. air-water interface, water-oil interface, etc.).

LITERATURE REVIEW

Silk fibers produced by silkworms are widely used in our daily life. While they have occupied an important niche in the textile industry for thousands of years, their potential as biomaterials has been recognized and developed only over the past decade[1]. Being non-toxic, non-immunogenic, and biocompatible with a broad range of animal species has allowed for the adherent properties of silk fibroin and silk-like proteins to be exploited for biomedical purposes. To date, silk fibroins have mainly been applied to wound healing, successfully performing as man-made blood-vessels[2], surgical sutures[3], and repair materials[4]. New processing strategies for silk fibers and proteins have expanded the biomedical utility of these molecules. For example, the gel spun silk-based matrix derived from silk fibroin was successfully applied for bladder augmentation in a murine model[5]. More recently, scientists determined that the cocoons from *Bombyx mori* harbor antioxidant and hypolipidemic properties and that the crude silk extracts have bioactivity against hypercholesterolemia and atherosclerosis[6].

Recently, the regenerated silk fibroin has been proved as an attractive candidate of a carrier for drug or therapeutic proteins delivery and is the focus of much ongoing research. Attachment of bioactive molecules or therapeutic proteins to silk fibroin has many benefits to enhance the properties of bioactive molecules in solution for delivery both *in vitro* and *in vivo*, including the therapeutic efficacy in the body, thermal stability, storage stability, and lengthens the circulatory half-life and decreases immunogenicity and antigenicity[3]. For instance, bioconjugations of insulin, glucose oxidase, L-asparaginase (L-ASNase), lipase and phenylalanine ammonia-lyase with the regenerated silk fibroin greatly improved their biological stability, reduced the immunogenicity and toxicity of the drug[7, 8, 9, 10, 11]. Moreover, The SELP (silk-elastinlike protein polymer)-controlled gene delivery approach could potentially improve activity of adenoviral-mediated gene therapy of head and neck cancer and limit viral spread to normal organs at the same time[12].

It has been known that the properties of silk-matrix are controlled by a combination of the chemistry and the spinning process, which directly affect the activity and stability of the enzymes attached. Spinning conditions, such as temperature, drawing rate, time, and specific type of silkworm, can modulate biomaterial features. In addition, chemistry, such as ion concentration, type of ion, and solution pH, can also affect the mechanical properties of silk fibroins[1]. In previous studies, degummed fibroin has generally been treated with aqueous solutions of hexafluoro-isopropanol (HFIP)[13], methanol[8], $CaCl_2$-ethanol[7, 9], or $Ca(NO_3)_2$-methanol[14]. Lu et al. has reported glucose oxidase attached to the regenerated silk fibroin film without treated with methanol remain more activity but lower stability than that treated with methanol[8]. After cross-linking L-ASNase with regenerated silk fibroin prepared with concentrated $CaCl_2$ mixture solution with ethanol and water (1:2:8, mol), the immunogenicity and toxicity of the drug significantly reduced, and its circulatory half-life lengthened *in vitro*[9].

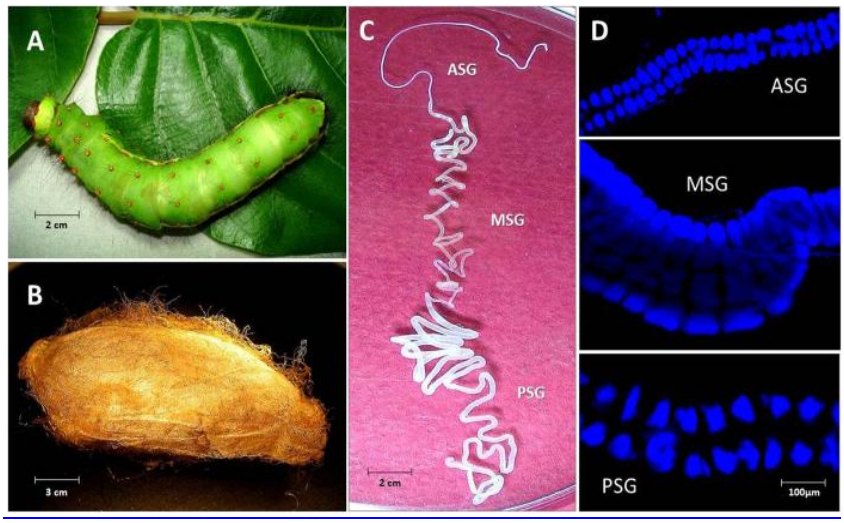
However, these studies have used only one treatment per experiment and, up to now, the systematic comparative analysis to distinguish the difference of those treatments has not yet been reported, thus we do not know which one is the best choice for future potential application. Here, we describe our systematic comparative analysis of silk fibroins prepared

with four of the commonly used preparative solutions, $Ca(NO_3)_2$-methanol, $Ca(NO_3)_2$-ethanol, $CaCl_2$-methanol, and $CaCl_2$-ethanol. The results could help to reveal the mechanisms of properties of silk-derived matrix under different treating conditions and provide evidence to choose right solution to prepare silk fibroins for potential drug delivery applications.

SILK GLAND STRUCTURE

Silk is synthesized in a pair of modified labial glands called silk glands. Each gland is composed of single-cell layered glandular epithelium in a long tubular structure enclosing a lumen made by stacking of just two secretory cells3. In *B. mori*, PSG is composed of about 525 secretory cells whose number remains constant, once determined in stage-25 of its embryogenesis16,17. The cell number is highly significant as it is a factor of its secretory activity. Silk glands of *A. assama* (Fig. 1A) secrete golden silk cocoon (Fig. 1B) at the end of larval stage. The ASG is about 5 cm long containing ~320 cells; MSG is about 10 cm long with ~550 cells, while the PSG is about 15 cm having ~800 cells surrounding luminal liquid silk (Fig. 1C,D). The PSG cells of *A. assama*, which are 35% higher than *B. mori* PSG, may cumulatively account for the larger cocoons in *A. assama* whose cocoon shell's mean weigh is about 600 mg, almost twice the mean weight of a typical *B. mori* cocoon shell.

A typical *B. mori* cocoon shell.



Antheraea assama (Saturniidae) larva, cocoon and silk gland

Silks are protein polymers produced by various species of insects and spiders. It is used for different purposes which include construction of protective shelter, structural support for developing eggs and egg sacs, reproduction, foraging and dispersal [1-3]. In the insect order Lepidoptera, Bombycidae and Saturniidae are the two important families utilized for commercial silk production. These two families are characterized by low silk production in early larval stage and enormous silk production in the last instars. During the production of cocoon, around 20% of the body mass is converted to silk. The tasar silkworm, Antheraea mylitta has the highest silk producing capacity among all silk spinning insects [4]. Silk glands in insect larvae are ectodermal in origin, which is anatomically and physiologically divided into three distinct regions, viz., anterior, middle and posterior

regions [5]. The anterior, middle and posterior region of silk glands of B. mori larvae consist of 200 cells, 255 cells and 520 cells respectively [6]. The morphogenesis [7] of silk glands are completed within eight days after egg laying. Silk is a natural fiber, up to 95% of which is composed of fibroin and sericin and the remaining 5% constituted by other proteins, waxes, fats, salts and ash [8]. Fibroin is the major structural protein formed by two different polypeptide chains, i.e., heavy (H) and light (L) chains of molecular weights 350 kDa and 25 kDa respectively. These two chains are linked together by di-sulfide bonds [3, 9]. A glycoprotein, P25, has also been associated with H-L complex by non-covalent interactions [10- 13]. In B. mori, fibroin was identified as the product of the posterior region of the silk gland, whereas sericin is produced in the middle region that serves as silk reservoir [14]. In the posterior region of silk gland the concentration of fibroin protein is around 12-15% by weight, while fibroin and sericin is 30% by weight in the middle region of silk gland [15]. Sericin accounts for 20-30% by weight of B. mori cocoon fibers [16, 17]. Sericins include sericin P (150 kDa), sericin M (400 kDa) and sericin A (250 kDa) identified in the distal, central and anterior of the middle regions of silk gland respectively [18]. In the lumen of gland silk proteins accumulate as a concentrated gel. During spinning, the liquid silk is subjected to stress and elongation, thus forming silk fibers. The unique properties of silk are mainly due to long storage of silk in the form of gel followed by its rapid conversion to silk filament [19]. Tasar, muga, eri, fagaria and shashe silks are produced by the non-mulberry silkworms A. mylitta, A. assama, Philosamia ricini, Attacus atlas and Gonometa postica respectively.

CONCLUSION

Full length fibroin gene sequence and its conceptually translated sequence were analysed to elucidate its coding, non-coding, intronic, and coding regions; within CDS, crystalline and amorphous regions. Codon preference for major amino acids was calculated on Sequence Manipulation Suite[48]. The sequence conservation among fibroin gene sequences of different moths was analysed both by manual pairwise alignment

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