The Mulberry Silkworm—A New Source of Bioactive Proteins

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Abstract: Beyond the commonly known proteins built silk fibres—fibroin and sericin, there are almost 300 bioactive proteins in the silkworm haemolymph. The aim of this work was to present bioactive compounds obtained from the silk fibre and isolated from the body of this insect, which may be used in medical and pharmacological applications. The most important are bioactive proteins. However, the juvenile stages of mulberry silkworm possess other very valuable active substances.

Key words: Bombyx mori L., fibroin, sericin, 1-deoxynojirimycin, haemolymph, silk fibre.

1. Introduction

The silkworms are a group of night moths that produce a silk fibre during their life cycle. The fibre produced by caterpillars adheres the old epidermis to bedding during moulting and serves for building cocoons, where the pupa transforms into a butterfly. The mulberry silkworm (Bombyx mori L., Bombycidae, Lepidoptera) is a domesticated insect bred by humans for about five thousand years due to its ability to produce one of the best natural silk fibres.

The wing span of this moth reaches 4 cm, but it has no ability to fly. After copulation, the female lays about 300-500 eggs of 1.5 mm in diameter. Multi-voltine races bred in tropical may produce 7-8 generations annually and the third generation of eggs hibernates until the next spring. Bivoltine breeds produce two generations annually and the third generation of eggs hibernates until the next spring.

The full cycle of development, starting from a caterpillar hatched from an egg to the butterfly, is controlled by two families of hormones: the juvenile hormones and the ecdysteroids. The presence of juvenile hormones in the haemolymph maintains the larval stage [1], during which a caterpillar feeds itself with mulberry leaves and grows through five instars (growth steps with four molting). At the end of the fifth instar, the level of juvenile hormones decreases in the haemolymph, and an increase of ecdysteroids, especially 20-hydroxyecdysone (20E), occurs and triggers the metamorphosis [2].

The aim of the work was to present bioactive compounds obtained from silk fibre and isolated from the body of the mulberry silkworm, which are used in medicine, pharmacological applications and tissue engineering.

2. Silkworms Bioactive Proteins

These days, the mulberry silkworm is used as a model system to study the various mechanisms. Bombyx mori has an estimated haploid nuclear genome of size 530 Mb broken into 28 chromosomes.
This moth was the first within the order Lepidoptera, for which a draft of the genomic sequence [4, 5] and the genetic variation map [6] were completed. It is believed to first be a central model for Lepidoptera genomics and genetics, and second only to fruitfly (*Drosophila melanogaster*) as an insect model for studies, which help elucidate the function of gene homologs and facilitate studies of insect domestication, morphogenesis, endocrinology, behaviour and immunity [3].

### 2.1 Silk Fibre Proteins

A large number of bioactive compounds may be isolated from the body of this insect for study of the biological activity of various synthetic compounds and biomedical and pharmacological properties. *Bombyx mori* is a main source of fibroin and sericin—two proteins of silk fibre, which are used in medicine for producing a biocompatible biopolymers for tissue engineering, biomedical and biotechnological applications and regenerative therapy [7-10]. Fibroin is a heterodimeric protein with a heavy chain (395 kDa) and two small subunits, P25 and L-chain of 25 kDa [8, 11]. The amino acid composition of silk fibroin from *Bombyx mori* consists primarily of glycine (43%), alanine (30%) and serine (12%) [8, 12]. Sericin comprises different polypeptides ranging in weight from 24 kDa to 400 kDa and is characterized by unusually high serine content (40%) along with significant amounts of glycine (16%) [8, 11].

Innovative fibroin biomaterials, such as hydrogels, films, non-woven silk mats, porous silk sponges, screws, scaffolds and plates, are widely used in orthopedics, craniofacial surgery, dental applications, reconstruction of damaged vessels and nerves by injury or disease [8, 13-16]. Silk fibroin has been also investigated for many other biomedical applications, including osteoblast, hepatocyte and fibroblast cell support matrixes, as well as for ligament tissue engineering [17], urologic tissue engineering and functional tissue engineering of the corneal epithelium [18, 19]. Moreover, cells, antibiotics, monoclonal antibodies and peptides have been encapsulated in silk using various processing approaches and material formats [20].

Sericin has excellent antibacterial, antioxidant, anticancer and UV-protecting activities. Moreover, this protein enhances the attachment of primary cultured human skin fibroblast. Thus, a sericin cream is used as an agent in treatment of difficulty to heal wounds, without causing allergic reactions [21]. Sericin has many other medical applications, such as anticancer drugs and anticoagulants. Moreover, this protein is useful in cell culture and tissue engineering of keratinocytes and fibroblasts [22]. Innovative sericin-based biomaterials for skin tissue repair (wound dressings), bone tissue engineering and micro-particles for drug delivery were also studied [22].

Study of the macrophage response showed that sericin does not manifest inflammatory activity when present in soluble form [8]. However, in the presence of lipopolysaccharide, sericin shows inflammatory response by initiating the release of tumor necrosis factor (TNF) [23]. Sericin is also used very widely in cosmetic industry. This protein enhances the elasticity of skin and has anti-wrinkle and anti-aging effects [22]. Sericin gel shows excellent moisturizing effects, because serine is the main amino acid of natural moisture factor (NMF) and prevents transepidermal water loss from the skin. Thus, dermatitis and other skin problems can be controlled with this protein. Moreover, sericin is added to nail cosmetics to prevent nail brittleness and impart nail gloss; and to hair and bath cosmetics to keep skin moisture and reduce damage to the hair surface [8]. It was also reported that the sericin peptides may be conjugated covalently with insulin. Innovative sericin and insulin bioconjugates exhibit a higher and longer-lasting hypoglycaemic activity than that of the native insulin [24]. Moreover, sericin as hydrophilic natural polymer can be exploited for conjugation with L-asparaginase.
to overcome the low water solubility of some drugs [8]. New silk biomaterials and biodegradable polymers are also combined to strengthen ceramic bone-scaffold materials made of calcium phosphate [25, 26]. The silk biomaterials are folded in complex ways that give it unique properties of both exceptional strength and versatility. Moreover, they can stabilize and deliver bioactive components or antibiotics to prevent infection, pharmaceuticals to enhance bone regrowth and support other therapies. They maintain structural stability under very high temperatures and withstand other extreme conditions, and they can be easily sterilized by autoclaving, ethylene oxide, γ-radiation or 70% ethanol [27]. Finally, the sericin gene and its mechanism of expression may be used for the transgenic silkworm production, that secretes biologically active recombinant fusion proteins of sericin and other therapeutic proteins (bioactive proteins for therapeutic application) [8, 22].

2.2 Haemolymph Proteins

A huge amount of highly valuable bioactive proteins may be also found in haemolymph of silkworm caterpillars. The number of proteins ranges from 241 to 298 [28]. However, still few of them have been well-characterized and the number of silkworm protein structures in the protein data bank (PDB) is very low [29]. In 2013, only 61 structures of silkworm proteins were deposited in the PDB. Among the haemolymph proteins, two major groups can be distinguished, i.e., high molecular weight storage proteins (SP, hexamerins of a molecular weight of about 500 kDa) and 30-kDa lipoproteins (LPs). Protein expression is at the highest level during the fifth larval instar, and many of important proteins are secreted to haemolymph then. Protein synthesis is connected to the disappearance of the juvenile hormones from the haemolymph [30].

Three storage proteins SP1 [31], SP2 [32] and SP3 [33] are very important for regular development of butterfly. The main physiological role of these proteins is the storage of amino acids at the final developmental stages of adult [34]. SP1 and SP2 are often also called as sex-specific proteins, because during the last instar, SP1 is expressed only in females [31] and SP2 expression is two times higher in females than in males [32]. The proteomic studies revealed that SP1 and SP2 constitute 60% of total fat body protein in females, whereas only 20% in males [33].

SP1 and SP2 are hexamerins and contain six subunits of a molecular weight of about 85 kDa. They were always described as homohexamers, however, the crystallographic studies carried out on proteins isolated from silkworm haemolymph revealed that SP2 and SP3 are present in silkworm body in the form of heterohexamer, which is composed of three SP2 and three SP3 chains [35]. The biological role of SPs is strictly dependent on their primary structure. Arylphorins are silkworm storage proteins. They serve as the main supply of nitrogen during the pupal stage [36].

The largest group of haemolymph proteins are lipoproteins (molecular weight 30 kDa) involved in lipid transport. The mulberry silkworm was the first Lepidoptera, in whose body the 30-kDa LPs were discovered [37]. Haemolymph lipoproteins transport lipids released from the silkworm fat body. They serve as storage proteins during pupation and adult development [38]. The 30-kDa LPs are used as the source of nutrition after enzymatic digestion [33]. Moreover, 30-kDa LPs are probably also involved in immune response pathway, namely antifungal defence system, because they are able to specifically bind glucose and glucans, which are the main components of fungal cell walls [39, 40]. What is more, it was reported that a member of the 30-kDa LPs family activates a prophenoloxidase cascade, the immune response pathway [40].

Silkworms haemolymph contains a number of different chemical compounds, and it might be that 30-kDa LPs are able to interact with them [41]. It was
reported that the 30-kDa LPs contain binding cavities for lipids, carbohydrates and maybe for other unknown compounds. Bmlp3 and Bmlp7 were used for the analysis and four potential binding cavities were found. Two pockets are located in the C-terminal domain (CTD) and one in the N-terminal domain (NTD), one between the NTD and CTD [42, 43].

The 30-kDa LPs might be capable of interacting with other proteins, especially proteins involved in the apoptosis process. This activity of Bmlp7 and Bmlp3 might be related to the NTD [42, 43]. The addition of silkworm haemolymph to cell cultures inhibits apoptosis and improves viability of the cells. To this date, anti-apoptotic properties of haemolymph were proven for insect, Sf9 [44-47], mammalian, CHO [48, 49] and human cell lines, HeLa [50] and HEK293 cell line [48]. Additionally, apoptosis inhibition by haemolymph resulted in a five-fold increase of erythropoietin production in the CHO cell culture [49].

Silkworm haemolymph could be efficiently used for apoptosis inhibition in commercial cell cultures and improve their productivity [50, 48]. Silkworm haemolymph could serve as a medium supplement and replace commonly used fetal bovine serum [51]. Moreover, silkworm haemolymph does not interact with viruses [50], which are often used as infective expression vectors for the production of recombinant proteins in cell cultures [52, 53]. Finally, some haemolymph proteins could be used in the future to treat diseases related to hyperactive apoptosis [48].

Moreover, it is plausible that the 30-kDa LPs are also capable of binding carbohydrate and this particular domain could be directly involved in immune response to fungal infections via β-glucan recognition. The CTD has a β-trefoil fold, suggesting a role of Bmlp7 in sugar binding and in the immune response to fungal invasion [42].

Recently, the unexpected identification of a cadmium binding site in the Bmlp7-I (Cd) structure suggested that Bmlp7 may be involved in a silkworm detoxification mechanism related to heavy metal pollution [42]. The link between the unexpected cadmium presence in the Bmlp7-I (Cd) and the biological sense of this discovery was established on the basis of reports about the silkworm ability to bio-accumulate heavy atoms [42]. Especially, the fifth instar larvae is able to ingest mulberry leaves with very high cadmium content [3].

Cell-penetrating properties of 30-kDa LPs made them capable to penetrate into various types of living cells via a receptor-independent endocytosis. They could be used for the delivery of biologically active proteins, DNA and other compounds into cell cultures and animal models in vivo [54, 55]. Therefore, members of the 30-kDa LP family are potential medicinal tools for cargo molecule delivery into target tissues [56].

3. Other Use of the Mulberry Silkworm

The mulberry silkworm is also used in other interesting ways. These days, silkworms pupae are used as a source of 1-deoxynojirimycin (DNJ), which is a potent α-glucosidase inhibitor [57]. What is interesting, innovative adsorbent based on silkworm chrysalides was prepared for heavy metals (Pb2+, Cu2+ and Ni2+) removal from wastewaters, found in the wastewater streams of industrial processes and agricultural areas [58]. It is also a good model organism for pest control studies [59] and a bioreactor for recombinant eukaryotic protein production [60]. The long-term expression of a human recombinant proteins in the silk glands had been demonstrated following gene delivery with a recombinant baculovirus [61, 62]. The protein production capacity of mulberry silkworms exceeds that of any other industrial system. Moreover, the glands of caterpillars produce an almost pure product. Thus, purification of recombinant protein from cocoon is a rather simple process. It was reported that 5 kg of pure collagen can be produced on a surface area of 300 m² with five workers caring for 1.5 million transgenic silkworms.
Moreover, the mulberry silkworm is a good model, which is consistent with the murine model, for development of drugs against Staphylococcus aureus infection [64], as well as for the evaluation of therapeutic drugs for hyperuricemia and gout (podagra) treatment [65].

4. Conclusions

The mulberry silkworm is used by man for centuries. Initially, in ancient China, silk glands of this insect were used for the production of strings for musical instruments and fishing lines. Later, for five centuries, silk worm was used for the production of silk fibres. Nowadays, innovative silk biomaterials and bio-structures are prepared for medicine and biotechnology purposes. Therapies may be easier and faster with using of silk micro-particles for drug delivery. It appears that this insect is valuable to humans not only by the wonderful fibres but also by the bioactive compounds, by which we develop new opportunities in various fields of science.

References


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