New protein studies in Polish sericulture

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Active compounds from silkworm

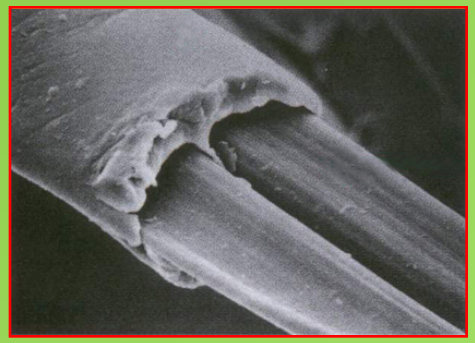
Silk fibre proteins:
- fibroin (70-75% of fibre)
- sericin (25-30% of fibre)

Proteins form the hemolymph:
- lipoproteins 30 KDa
- storage proteins 500 kDa

Transgenic silkworms:
- collagen, albumins,
- spider silk,
- mouse antibody,

DNJ (1-deoxynojirimycin)

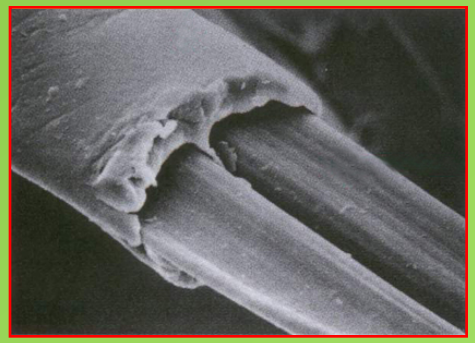
Silk fibre proteins



FIBROIN:

- an insoluble center of the silk,
- heterodimer with a heavy chain (395 kDa) and two small, subunits: P25 and L-chain, of 25 kDa,
- glycine (43%), alanine (30%) and serine (12%),
- biomaterials: hydrogels, films, non-woven silk mats, tubes, porous silk sponges, screws, scaffolds, plates and microsphares,
- orthopedics, craniofacial surgery, dental applications, to repair broken bones, reconstruction of damaged vessels and nerves,
- biomedical applications - osteoblast, hepatocyte and fibroblast cells support matrixes,
- ligament and vessel tissue engineering,
- micro- and nanospheres to encapsulate and release growth factors, small molecules or therapeutic compounds.

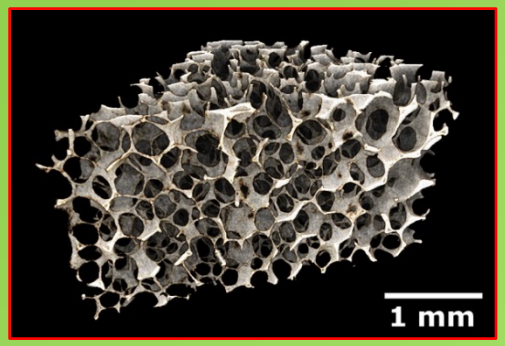
Silk fibre proteins



FIBROIN BIOMATERIALS:

- silk and polymers,
- biocompatible and biodegradable,
- combined to strengthen ceramic bone-scaffold materials made of calcium phosphate,
- folded in complex ways - unique properties of strength and versatility,
- deliver bioactive components or antibiotics to prevent infection, pharmaceuticals and other therapeutics to support,
- structural stability under very high temperature and other extreme conditions,
- easily sterilized by autoclaving, ethylene oxide, γ-radiation or 70% ethanol,
- do not manifest inflammatory activity and do not induce an immune response.

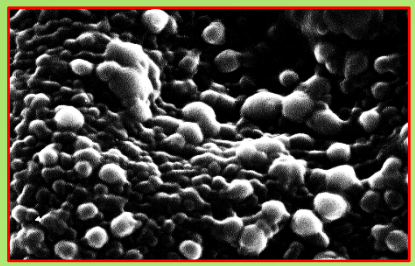
Fibroin biomaterials



silk scaffold

silk palatal plate

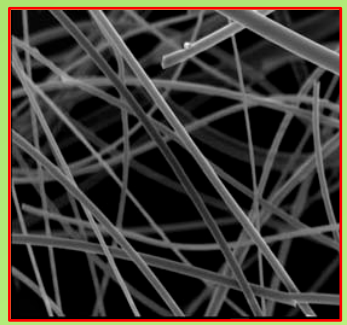
silk sponge



silk microphases

silk screws and ties

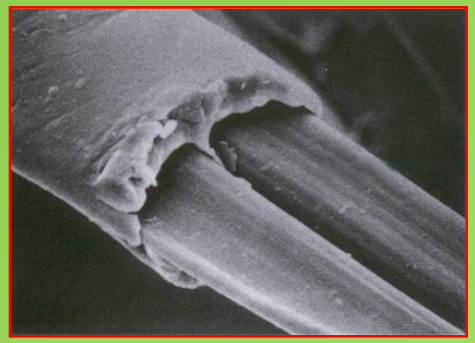
silk film



silk fibres

silk vessel

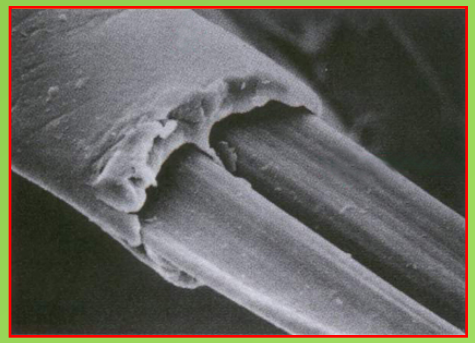
Silk fibre proteins



SERICIN:

- soluble silk glue,
- various polypeptides from 24 to 400 kDa,
- characterized by unusually high serine content (40%) - natural moisture factor (NMF),
- antiwrinkle and antiaging effects,
- excellent antibacterial, antioxidant, anticancer and UV-protecting activities,
- sericin cream used in treatment of difficult to heal wounds (without causing allergic reactions),
- dermatitis and other skin problems,
- moisturizing effects, prevent transepidermal water loss from the skin,
- body, nail and hair cosmetics to moisture skin and reduce damage to the hair surface,

Silk fibre proteins

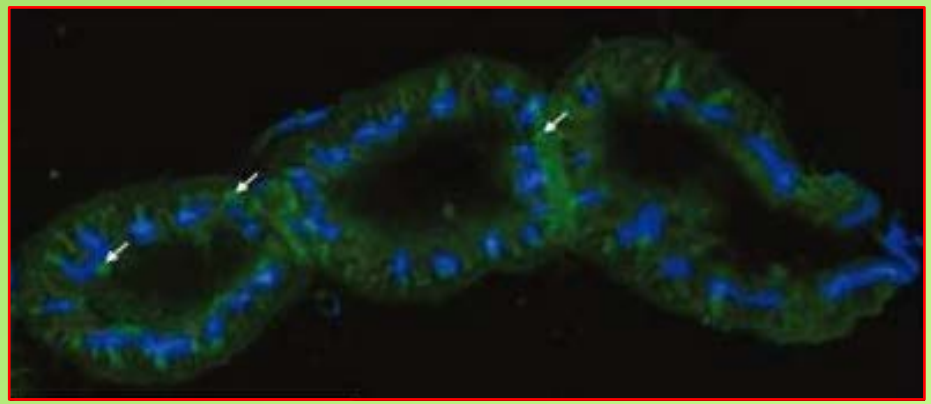


SERICIN:

- sericin and insulin bioconjugates exhibit a high and long-lasting hypoglycemic activity,
- accelerates proliferation of various mammalian cells,
- medical applications - anticancer drugs and anticoagulants,
- hydrophilic natural polymer overcomes the low water solubility of some drugs,
- the sericin gene and its mechanism of expression may be used for the transgenic silkworms production, that secrete bioactive recombinant fusion proteins of sericin and other therapeutic proteins (bioactive proteins for therapeutic application).

Proteins from hemolymph

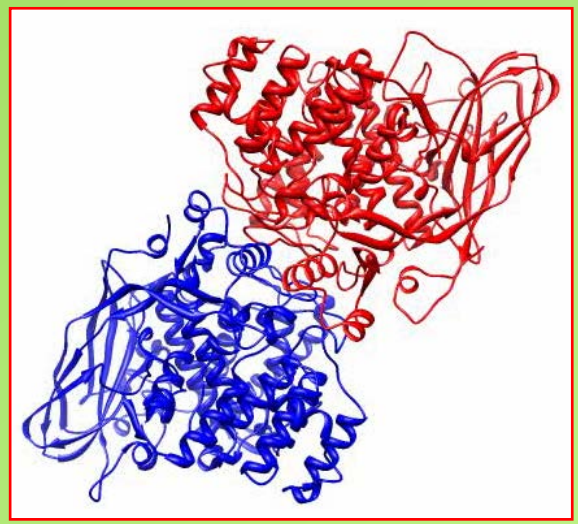
- number of proteins 241 to 298,
- only 61 are well-characterized and deposited in the Protein Data Bank (PDB),
- 500 kDa storage proteins SP1, SP2, SP3 and 30-kDa lipoproteins (LPs).



Blue fluorescence - the nuclei.
Green fluorescence - the proteins.

Storage proteins SP

- heterohexamer composed of three SP2 and three SP3 chains,
- biological role - the main supply of nitrogen and amino acids during the pupal stage,
- the highest level of protein expression during the fifth larval instar,
- SP1 and SP2 constitute 60% of total fat body protein in females, 20% in males.

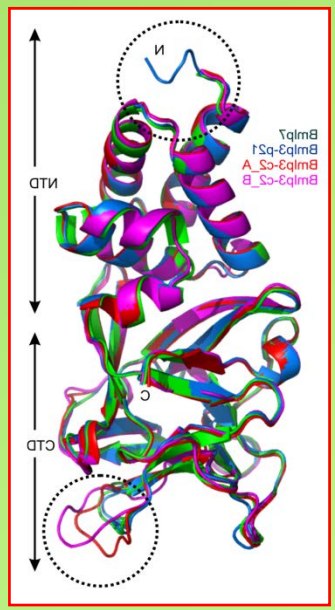


SP2-SP3 complex

Lipoproteins

- transport lipids released from the silkworm fat body,
- storage proteins during pupation and adult development,
- biological role - immune response - antifungal defense system, (specifically bind glucose and glucans - the main components of fungal cell walls),
- are able to interact with lipids, carbohydrates and other chemical compounds from hemolymph – 4 binding cavities in Bmlp3 and in Bmlp7.

Bmlp3

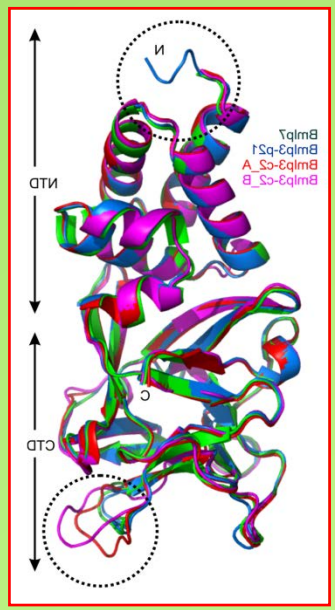


Bmlp7

Lipoproteins

- N-terminal domain interact with proteins involved in the apoptosis,
- the addition of silkworm hemolymph to cell cultures inhibits apoptosis and improves viability of the cells,
- hemolymph does not interact with viruses, used as infective expression vectors for the production of recombinant proteins in cell cultures,
- could be used to treat diseases related to hyperactive apoptosis,
- C-terminal domain binds glucan (immune response to fungal invasion).

Bmlp3

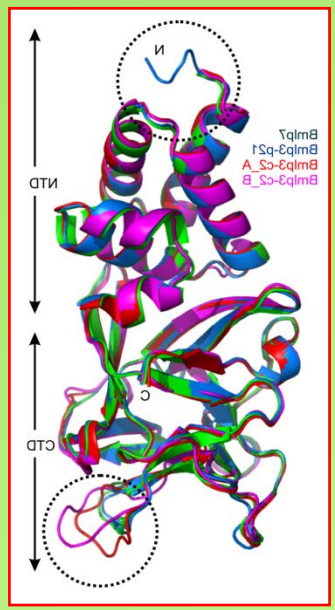


Bmlp7

Lipoproteins

- cadmium binding sites - detoxification mechanism related to heavy metal pollution - the silkworm ability to bioaccumulate heavy atoms,
- cell-penetrating properties made them capable to penetrate into living cells via a receptor-independent endocytosis,
- used for the delivery of bioactive proteins, DNA and other compounds into cell cultures and animal models *in vivo*,
- are potential medicinal tools for cargo molecule delivery into target tissues.

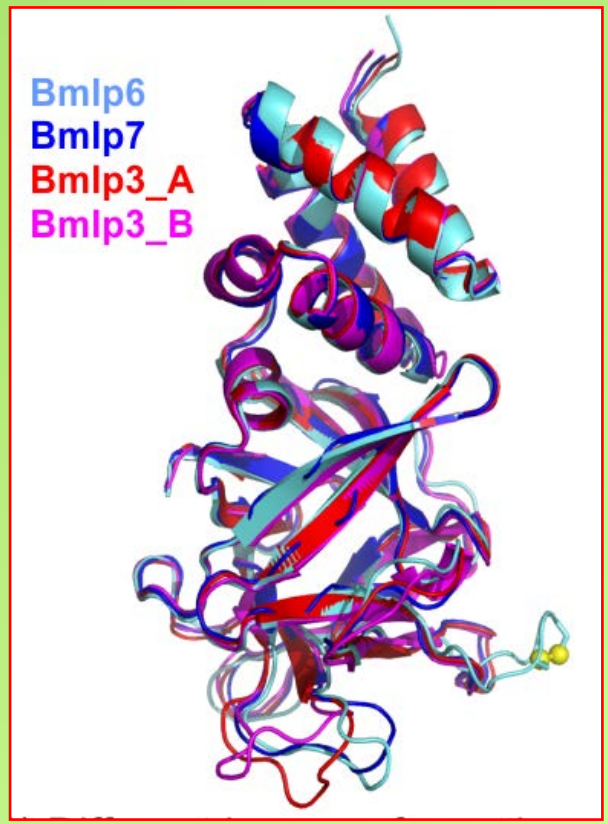
Bmlp3



Bmlp7

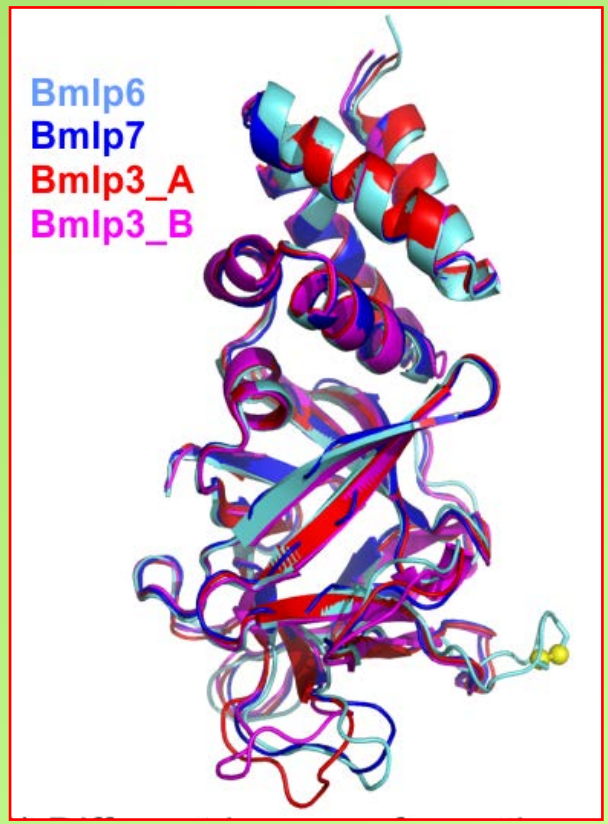
Lipoproteins

- the silkworm genome contains 46 genes of LPs (24 are classified as typical 30-kDa LPs) ,
- Bmlp3, Bmlp6 and Bmlp7 share a similar fold (it is very likely all 24 typical LPs have the same fold),
- many differences:
  1. different conformation of CTD loops,
  2. different electrostatic surface potential,
  3. different potential binding cavities.



Lipoproteins

- LPs interact with other proteins and carbohydrates,
- the structural differences enable them to recognize different binding partners,
- the high number of “isoforms”,
- different ligand specificity of each LPs,
- it might be speculated that the proteins play in the insect immune response a role similar to human antibodies.



References

1. Pietrzyk A.J., Bujacz A., Lochynska M., Jaskolski M., Bujacz G. (2011). Isolation, purification, crystallization and preliminary X-ray studies of two 30 kDa proteins from silkworm hemolymph. *Acta Cryst.* F 67, 372-376.
2. Pietrzyk A.J., Panjikar S., Bujacz A., Mueller-Dieckmann J., Lochynska M., Jaskolski M., Bujacz G. (2012). High-resolution crystal structure of *Bombyx mori* lipoprotein 7: crystallographic determination of the identity of the protein and its potential role in detoxification. *Acta Cryst.* D 68, 1140-1151.
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5. Pietrzyk A.J., Bujacz A., Lochynska M., Jaskolski M., Bujacz G. (2014). Crystal structure of *Bombyx mori* lipoprotein 6: comparative structural analysis of the 30-kDa lipoprotein family. PLoS ONE 9(11): e108761. doi:10.1371/journal.pone.0108761.


Thank you
for attention