Feature Article

Polymeric materials based on silk proteins

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ABSTRACT

Silks are protein-based fibers made by arthropods for a variety of task-specific applications. In this article, we review the key features of silk proteins. This article initially focuses on the structure and function of silk proteins produced naturally by silkworms and spiders, followed by the biological and technical processing of silk proteins into a variety of morphologies (including capsules, fibers, films, foams, gels and spheres). Finally, we highlight the potential applications of silk-based materials.

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1. Introduction – nature's biopolymers

Biopolymers such as polyamino acids, polynucleic acids, polyphenols and polysaccharides have evolved over billions of years to carry out a myriad of tasks such as catalysis, molecular recognition or the storage of energy or information. Biopolymers are synthesized from a very limited number of building blocks and it is their complex three-dimensional structures that are responsible for their highly specialized properties [1–3].

Polyamino acids and polynucleic acids are synthesized in template-directed catalyzed reactions yielding monodisperse linear polymers composed of a specific sequence of monomers; whereas polyphenols and polysaccharides are prepared via untemplated catalyzed reactions yielding polydisperse polymers with a wide variety of potential structures (including linear and branched) depending upon the monomers involved [4–9].

Biopolymers are commonly occurring structural elements of biological systems. Polysaccharides are the most abundant biopolymers on earth, cellulose and chitin serve as structural elements in plant cell walls and animal exoskeletons; polyphenols such as lignins are important structural elements in wood and other plants; and polyamino acids such as collagen and elastin are the main components of blood vessels, connective tissues and skins in animals and humans [4,5,9–20].

Silkworms produce polyamino acid-based (silk protein) cocoons to protect themselves during their metamorphosis into moths, and humans have harvested silk fibers from these cocoons for centuries to produce textiles due to their characteristic luster, moisture absorbance and strength [21,22]. Web-weaving spiders produce silk-based webs (from a variety of different silk protein-based fibers) to capture prey; certain of these silk fibers have mechanical properties surpassing Nylon, Kevlar, silkworm silk, and high-tensile steel [23–26], as summarized in Table 1 [27]. In this article, we will focus on materials derived from silk proteins produced by silkworms and spiders.

1.1. Protein characteristics and structure

Polyamino acids (known as polypeptides or proteins) in higher organisms are synthesized from combinations of up to 19 amino acid monomers ($-NH-CHR^1-CO-$) and 1 imino acid monomer ($-NR^1-CHR^2-CO-$), linked via amide bonds (also known as peptide bonds) between the monomers (which are more commonly referred to as residues). In higher organisms, only the L-amino acids are used as monomers, whereas in lower life forms (such as bacteria or lower plants) D-amino acid monomers can also be incorporated [8,28,29].

In vivo, polyamino acids are synthesized in a template-directed fashion: first, DNA is used as a template in the synthesis of messenger RNA (mRNA) via a process known as transcription; mRNA can subsequently be used as a template by ribosomes in the synthesis of a sequence-specific polypeptide, this process is known as translation, because the information stored in a polynucleic acid (genetic code) is translated into information in a polyamino acid (functional code) [30,31].

The sequence of residues in the polypeptide is known as the primary structure. The amino acid residues display different functional groups on the polyamide backbone of the polymer; these functional groups can be categorized as polar, non-polar, aromatic,

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Table 1
Mechanical properties of silk and man-made fibers

Material	Strength, σ_{max}/GPa	Extensibility, ε_{max}	Toughness/MJ m^{-3}
B. mori silk	0.6	0.18	70
A. diadematus MA silk	1.1	0.27	160
A. diadematus Flag silk	0.5	2.70	150
Nylon	0.95	0.18	80
Kevlar 49™	3.6	0.027	50
High-tensile steel	1.5	0.008	6

anionic or cationic. After polymer synthesis, supramolecular interactions (such as hydrogen bonding between the amide bonds in the backbone of the polymer, or π interactions between aromatic groups) determine the local conformation of the polypeptide which is known as the secondary structure – prominent examples of common secondary structures are: α-helices, β-sheets and β-turns (see Fig. 1) [2,32].

Hydrogen bonds between the hydrogen atom attached to the nitrogen atom of an amide and the carbonyl oxygen atom of the fourth amino acid on the amino-terminal side of the peptide bond encourage the polymer to coil around an axis into an α-helix; each helical twist contains on average 3.6 amino acids and is 5.4 Å in length. α-Helix formation is encouraged by ion pair formation between oppositely charged residues 3 or 4 amino acids apart, and π-interactions between similarly spaced aromatic amino acids [2,32]. Less common are helical twists containing 3 amino acids (known as 3_1- or 3_{10}-helice [33,34]) which are important in some silk proteins [35–37].

In certain cases, hydrogen bonding between chains (intrachain or interchain) that are side by side causes the polypeptide chain to adopt a zigzag conformation, known as a β-sheet. Amino acids with small side chains such as glycine and alanine allow stacking of β-sheets, whereas bulkier amino acids discourage this sort of assembly process [2].

Turns and loops are also frequently occurring secondary structures in polypeptides. Particularly common are 180° loops, known as β-turns, which consist of 4 amino acids where the carbonyl oxygen of the first amino acid is hydrogen bonded to the hydrogen on the amine of the fourth amino acid. Importantly, the second and third amino acids do not participate in hydrogen bonding [32].

Polypeptides therefore contain regions that are either locally disorganized or locally organized dependent upon their primary structure, and covalent or non-covalent cross-links between different regions within a polypeptide chain determine the overall three-dimensional arrangement of the polypeptide chain, which is known as the tertiary structure. Further interactions (covalent or non-covalent) between individual polypeptide chains (identical or different) determine a protein's quaternary structure [3,38].

The process by which polypeptides assume their secondary, tertiary and quaternary structures after polymerization is known as 'folding' [38,39] and is in some cases aided by accessory proteins. Once the process of folding is complete and the polypeptides are fully assembled into their biologically active conformations, the polypeptides are said to be in their 'native' state [39].

1.2. Silk proteins – structure and hierarchical assembly

Silks are protein-based fibers made by arthropods for a variety of task-specific applications. Silk fibers are typically composite materials formed of silk protein and other associated molecules such as glycoproteins and lipids [40–44].

1.2.1. Silkworm silk

Of all natural silk-producing animals, mulberry silkworms (*Bombyx mori*) are of the most economic importance, because it is possible to rear them in captivity. After the eggs laid by adult moths hatch, the caterpillars are fed fresh mulberry leaves for a month until they are mature enough for metamorphosis into a moth, which requires the construction of a protective cocoon of silk [45–48]. Once the cocoon is complete, silk farmers kill the caterpillar via exposure to hot water/steam, and the silk can be harvested. The crude silk needs both degumming [49–53] (from the glue-like sericin) and processing before it can be dyed and sold.

The fact that there is a readily available source of silkworm silk has facilitated an understanding of its structure and function [22,44,54,55]. *B. mori* silk has a core–shell type structure, with its core composed of a complex of 3 proteinaceous components: a large protein, known as heavy chain fibroin (*H-chain*, ca. 350 kDa) that is linked to a second small protein, known as light chain fibroin (*L-chain*, ca. 25 kDa) via disulfide bonds; and a third small glycoprotein, known as the P25 protein (*P25*, ca. 30 kDa) is associated via non-covalent hydrophobic interactions [56–58]. The molar ratios of H-chain:L-chain:P25 are 6:6:1; the H-chain is hydrophobic and provides crystalline like features to the silk thread, whereas the L-chain is more hydrophilic and relatively elastic, and the P25 protein is believed to play a role in maintaining the integrity of the complex [56–59]. Before fiber formation, a solution of the three proteins is secreted from two glands within the silkworm, assembling into twin filaments that emerge from an exit tube in its head (known as the spinneret) and dry upon exposure to air. The resulting core contains anisotropic β-sheet-rich nanocrystals that are loosely aligned with the fiber axis and dispersed in an unstructured matrix [21,54,55,59,60]. Another pair of glands secretes glue-like **sericins** [61–64] (a set of serine-rich glycoproteins) that coat the fibroin filaments and ensures the cohesion of the cocoon by sticking the twin filaments together. Finally the fiber is coated with a variety of other proteins postulated to protect the cocoon against microbes and predators (see Fig. 2) [65–73].

1.2.2. Spider silks

Spiders have evolved to be able to produce a variety of task-specific silks for catching prey (via trapdoors and webs), protection and preservation of their offspring and prey (in cocoon-like structures), as a lifeline to escape from predators and even as a means of transport (parachute-like structures) [23,74–81].

More than 34,000 different species of spiders have been identified, of which approximately half catch their prey in webs (of which there are over 130 catalogued designs). Orb webs are a particularly interesting example of web design and they are constructed from four different types of task-specific silks (see below) [23,24,76,82,83].
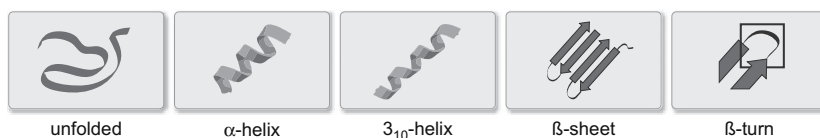

Fig. 1. Common secondary structural motifs in proteins.