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Silk sericin and its applications: A review

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Silk consists of two types of proteins, silk fibroin and sericin. Sericin contributes about 20-30 per cent of total cocoon weight. It is characterized by its high content of serine and 18 amino acids, including essential amino acids. There are different methods of isolation of sericin from silk thread. Solubility, molecular weight, and gelling properties of sericin depend on the method of isolation. It has wide applications in pharmaceuticals and cosmetics such as, wound healing, bioadhesive moisturizing, antiwrinkle and antiaging.

Keywords: Silk, Silk proteins, Sericin, Isolation

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Introduction

Sericulture in India

Silk has been a scientific curiosity for centuries and a new insight about these polymers are surfacing with improved analytical methods and the tools of molecular biology. Silk includes a broad range of primarily protein-based high molecular weight polymers often associated with insects, silkworm, and orb weaving spiders[1]. Sericulture, in India, is essentially a cottage industry. The post rearing operations are fairly cost-effective and silkworm rearing is still only considered as a side activity to the main farm activity. India is the second largest producer of silk in the world and has the distinction of producing all the four varieties of silk. Presently, India produces nearly 16,700 mt silk/y and reeled silk prices are in the range of Rs 900-1300/kg, the pierced cocoons and wastesilk generated at the rearing are sold at Rs 80-100/kg. This waste contributes nearly 30 per cent of total cocoon production[2,3]. Wastesilk can be classified as waste from the cocoon, rearing waste, and thread waste. Silk wastes can be used as coarse yarn and spun silk, which can be incorporated in natural rubber to achieve the physicochemical properties[4]. It is also possible to utilize the silk waste by extracting fibroin and sericin from silk polymer, which helps to make sericulture a viable agro industry.

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Structure of Silk

Silk is a continuous strand of two-filaments cemented together forming the cocoon of silkworm, *Bombyx mori.* Silk filament is a double strand of fibroin, which is held together by a gummy substance called silk sericin or silk gum. Silk fibroin is the protein that forms the filament of silkworm and gives its unique physical and chemical properties[4,5]. Silk adapts various secondary structures, including α-helix, β-sheet, and crossed β-sheet[6].

Fibroin is a glycoprotein composed of two equimolar protein subunits of 370 and 25 kDa covalently linked by disulphide bonds. Fibroin filament is made of both crystalline and amorphous domains. The amorphous domains are characterized by the presence of amino acids with bulkier side chains, whereas the crystalline domains are characterized by high percentage of alanine, glycine, and serine (12, 30, 44 per cent, respectively), which contains short side chains to permit the close packing densities for overlying sheets[7]. The β-sheet form (silk II or β-silk) and crystalline form (silk I) have been reported for silk fibroin, having relative molecular masses of 350 – 415K (refs 3,8).

Anti-parallel β-sheet structure forming microfibrils is responsible for the crystalline nature of the silk fibre. The microfibrils are organized into fibril bundles, with several bundles leading finally to a single silk thread[7].

Sericin

Sericin is a second type of silk protein, which contains 18 amino acids including essential amino acids and is characterized by the presence of 32 per cent of serine. The total amount of hydroxy amino acids in sericin is 45.8 per cent. There are 42.3 per cent of polar amino acid and 12.2 per cent of nonpolar amino acid residues. Sericin contributes about 20-30 per cent of total cocoon weight. Their main role is to envelop the fibroin. In presence of sericin the fibres are hard and tough and become soft and lustrous after its removal. Sericin occurs mainly in an amorphous random coil and to a lesser extent, in a β-sheet organized structure. The randomly coiled structure easily changes to β-sheet structure, as a consequence of repeated moisture absorption and mechanical stretching[7,9].

Forms of Silk Sericin

Sericin can be classified into three fractions, depending on their solubility as sericin A, sericin B, and sericin C. Sericin A is the outermost layer and insoluble in hot water. It contains about 17.2 per cent of nitrogen and amino acids like, serine, threonine, glycine, and aspartic acid. Sericin B is the middle layer and on acid hydrolysis it yields amino acid of sericin A, in addition to tryptophan. It contains 16.8 per cent of nitrogen. Sericin C is the innermost layer, which is adjacent to fibroin and is insoluble in hot water and can be removed from fibroin by treatment with hot dilute acid or alkali. On acid hydrolysis it yields proline in addition to amino acids of sericin B. It also contains sulphur and 16.6 per cent of nitrogen [9,10]. Sericin has been divided into various species based on relative solubilities. Various fractions of sericin are also designated by other researchers depending on their dissolution behaviour as sericin A and B, or sericin I, II, III, and IV, or S1, S2, S3, S4, and S5, and as α, β, and γ modification[6,7]. The major molecular conformation of easily soluble sericin is random coil, whereas the β-sheet structure is more difficult to dissolve. The repeated moisture absorption makes molecular aggregation structure denser and forms more crystalline structure, which is having reduced solubility.

The γ-ray study shows the three layers in the sericin structure. The outer layer contained some fibre direction filaments, middle layer exhibits cross-fibre direction filaments, and the inner layer shows longitudinal filaments[11]. The structure of sericin also depends on the casting temperature. Lower the casting temperature more the sericin molecules assume β-sheet structure rather than random coil[12,13].

Properties of Silk Sericin

Gelling Property

Sericin contains random coil and β-sheet structure. Random coil structure is soluble in hot water and as the temperature lowers the random coil structure converts to β-sheet structure, which results in gel formation[14, 15].

Sol-Gel Transition

Sericin has sol-gel property as it easily dissolves into water at 50-60^{o}C and again returns to gel on cooling[16].

Isoelectric pH

As there are more acidic than basic amino acid residues the isoelectric point of sericin is about 4.0 (ref. 7).

Solubility of Sericin

Solubility of sericin in water decreases when the sericin molecules are transformed from random coil into the β sheet structure. The solubility of sericin increases by addition of poly (Na acrylate) and decreases by the addition of polyacrylamide, formaldehyde, or resin finishing agents[17-19].

Molecular Weight

Extracting sericin using 1 per cent sodium deoxycholate solution followed by precipitation, using equal volume of 10 per cent trichloroacetic acid, shows molecular weight in the range of 17100 to 18460 (ref. 20).

Extraction of sericin by hot water shows molecular weight of 24000 by gel electrophoresis, whereas spray-drying method produced sericin of molecular weight 5000-50,000, with enzyme action 300-10,000 and 50,000 when it is extracted with aqueous urea at 100ºC (ref. 21).

Isolation of Silk Sericin

Isolation with Aid of Heat

The removal of gum from crude silk is based entirely upon its solubility in hot water. The number of methods illustrated by researchers for removing gum are as follows:

- The removal of gum by dilute solution of sodium carbonate.
- Hot water extraction of raw silk, followed by evaporation to obtain powder.
- Boiling of the crude silk in water and renewing the water until the extract no longer gives a precipitate with gallic acid.
- Three successive 1 h extractions of silk or simply heating in water at 100°C or autoclaving at 118°C or autoclaving for 3 h under 2.5-3 atmosphere pressure.
- Sericin with average molecular weight of 50,000 extracted with aqueous solution of urea at 100°C from cocoons[21].
- Using water at 50-60°C for 25 d to avoid the decomposition.[22]
- Silk fibres can be completely degummed in boiling solutions of pH 11 containing 5-6 per cent bentonite[23].

In a series of experiments, it is demonstrated that most of the sericin is removed by autoclaving for one and half hour under pressure of 600-700 mm Hg (14 lb), whereas increasing the time of treatment to 2 h causes no greater loss in weight of fibres. It is also observed that the extracting sericin at low pressure (25 cm Hg, 5 lb) shows good results.

When sericin is extracted from cocoons of *Bombyx mori* by heating on water bath and autoclaving at different temperatures the satisfactory yield is obtained by autoclaving at 105°C for 30 min with good gelling property and yield. Further increase in temperature increases the yield but looses its gelling property[24].

Extraction of Silk Sericin using Enzymes

Extraction is carried out by using enzyme alkylase[25] or with 2-2.5g/L alkaline protease at 60°C for 90 min, at pH 10 (ref. 26).

Hydrolysis with trypsin at different concentrations, temperatures and treatment times is employed for extraction of sericin. For 1 per cent of trypsin solution the hydrolysis is almost complete in 10 and 32 h at 37 and 20°C, respectively. The amount of sericin obtained by 4 h treatment with 1 and 8 per cent of trypsin solution is 26.4 and 28.7 per cent, respectively[27].

Precipitation of Silk Sericin from Aqueous Solution

Several methods[22] proposed for precipitations of sericin from its aqueous solution are as follows:

- Precipitation of sericin with lead acetate from aqueous solution, which is decomposed by hydrogen sulphide and the protein is separated by alcohol.
- Evaporation of sericin solution and dissolution of obtained residue in alkali followed by precipitation by alcohol.
- Precipitation of sericin from aqueous extract by acetic acid and then treating the precipitate with alcohol and ether.
- According to Shelton and Johnson[22] the method of recovery by evaporation to dryness is least satisfactory. Protein molecule on continuous boiling of the aqueous solution in an open kettle loses its capacity to form gel. A method in which hot concentrated sericin solution is poured directly from the autoclave into 7 to 8 vol of 95 per cent alcohol is found to be satisfactory. The clear supernatant liquor is separated from the precipitate and the precipitate is washed with 95 per cent alcohol. The obtained cake is dried slowly over calcium chloride in a desiccator to get white and easily pulverized powder.
- Salting out of sericin solution by addition of 15 g solid ammonium sulphate to each 100 mL of solution results in gelatinous precipitate, which does not support bacterial growth.
- Hamaoka *et al.*[28] have extracted silk with hexane to remove oils and fats and by heating in water at 120°C for 30 min. After freeze thawing the sericin deposited in thawing is washed with hydrophilic organic solvent and the powdered sericin is recovered.

Applications of Silk Sericin

Silk sericin due to its proteinous nature is susceptible to the action of proteolytic enzymes present in body and hence it is digestible. This property makes it a biocompatible and biodegradable material. Because of some additional properties like, gelling ability, moisture retention capacity, and skin adhesion. it has wide applications in medical, pharmaceutical, and cosmetics.

Medical and Pharmaceutical Applications

Sericin is soluble in hot water and as the time precedes it converts into gel. Jun *et al.*[29] have found that conversion of α-random coil to β-sheet structure gives gel. One per cent aqueous sericin solution produces gel at pH 6-7 at room temperature and gelation speed increases as the concentration of sericin increases[30-32]. The aqueous sericin solution containing 1.5 and 2 per cent w/w of sericin obtained by autoclaving at 105°C for 30 min does not show good gelling. Sericin gel in the presence of glycerin, propylene glycol, and tween-80 shows synerisis, whereas sericin with pluronic and carbopol gives stable gels. In the presence of pluronic sericin gel it shows concentration dependance[24]. Kewon *et al.*[33] have shown the effect of concentration of pluronic and temperature on the gel property of sericin. The gelling of sericin is accelerated with increase in temperature and with increase in poloxamer concentration, whereas the sol-gel transition of sericin becomes irreversible. Blends of polyvinyl alcohol and sericin are cross-linked to give hydrogels. Hydrogels with good mechanical strength and water resistance are produced by casting aqueous solution containing sericin and dimethyl urea on a glass plate and heating at 80 and 120°C for 1 and 3 h, respectively[34].

Sericin gives a very stable emulsion when shaken with water immiscible liquid[22,35]. The sericin protein is also used as horizontal alignment film for the liquid crystal to achieve uniform optical properties and to increase the stability of product[36].

Fibroin and sericin, when sulphonated show anti-thrombotic effect[37]. One stage condensation of salicylic acid, formaldehyde, and sericin creates a co-polymer with a molecular mass of 6000-8000 Da. A concentration of 0.01-1 mg/mL in blood exhibits anti-coagulant, fibrinolytic, and anti-aggregation activity towards thrombocytes at 0.5 mg/mL (ref. 38). Sericin with molecular weight of 1,00,000 shows an inhibitory action for tyrosinase and lipid per oxidation with rat brain homogenates[39,40]. The addition of 0.1-2 mg/mL of sericin into the aqueous solution shows heat resistant DNA polymerase activity[41]. Sericin has been found to possess wound-healing property and can be used as wound healing covering material in the form of film[42]. Sericin also has adhesive property due to its chemical composition. It has affinity to keratin[7]. Silk threads obtained from mulberry silkworm can be used for making surgical sutures[43]. Silk sericin membranes are good bandage materials and the film has adequate flexibility and tensile strength. Due to its good biocompatibility and infection resistant nature, it is a novel wound coagulant material. Additionally, its flexibility and water absorption properties promote smooth cure for defects in the skin and do not cause any peeling of the skin under regeneration when detached from the skin[44].

Kurioka[45] has explained silk sericin as a biomaterial. The silk sericin has the potential to find application in the development of contact lenses. The graft polymers are prepared with methyl methacrylate or styrene and are also biocompatible[46,47].

Intake of sericin containing food relives constipation, suppresses development of bowel cancer and accelerates the absorption of minerals. In rats, consumption of sericin elevates the apparent absorption of zinc, iron, magnesium, and calcium by 41, 41, 21, and 17 per cent, respectively[48]. A dietary supplementation of 4 per cent of sericin suppresses induced constipation in rats because of its low digestibility along with water holding capacity[49]. Sericin, when given orally, causes a dose dependent decrease in the development of colonic aberrant crypt foci. The incidence and the number of colon tumours are suppressed by consumption of sericin. Sericin have anti-tumor activity[50-52].

Oxygen permeable membranes are made up of fibroin and sericin with 10-16 per cent water and are used for contact lenses, and as artificial skin[53]. Agar and/or compounds containing agarose and sericin are mixed with water to form sheet shaped gels and which when dried at 0-40°C under load of 0.01-2 kg/cm^2 give the polymer membranes[54].

Cosmetic Applications

In addition to above-mentioned medical and pharmaceutical uses of sericin, it has been used as component of cosmetics. Sericin alone or in combination with silk fibroin has been used in skin, hair, and nail cosmetics. Sericin when used in the form of lotion, cream and ointment shows increased skin elasticity, antiwrinkle, and antiaging effects[7,55-57].

Padamwar *et al.*[58] have shown the moisturizing property of the sericin gel, evaluated by hydroxyproline assay, impedance measurement, trance epidermal water loss (TEWL), and scanning electron microscopy (SEM). Sericin gels increase the hydroxyproline content in stratum corneum and decrease skin impedance, which reveals moisturizing property of sericin. Sericin gels with pluronic and carbopol, act as moisturizer by repairing natural moisturizing factor (NMF) as well as prevent TEWL by preventing water loss from the skin. SEM has shown the decrease cracking and flaking as compare to dry skin and normal skin replicas.

Powder containing 5-30 per cent sericin with average molecular weight 7,000-3,00,000 and 70-95 per cent silk fibroin when applied as film shows antistaticity and moisture absorbability[59]. Sericin hydrolysate solution shows that dermatitis is controlled[60]. Sweat and sebum absorbing type of cosmetics containing cellulose fibres impregnated with fibroin dispersion and aqueous sericin solution are also reported[61]. Lotion containing 1 per cent w/w sericin and 4 per cent w/w D-glucose shows moisturizing and conditioning effect[62]. Creams containing 0.001-30 per cent w/w of sericin have improved cleansing properties with less skin irritation[63]. Sericin powder in the form of sericin hydrolysate coated talc, mica, titania, iron oxide, and nylon have been used to formulate foundation cream and eyeliners[64]. The microcapsules or nanocapsules consisting of polysiloxane gel, UV absorbent core and UV scattering agent, silicone treated mica, silicon treated titanium dioxide, silicon treated iron oxides, squalene, glycerin trioctanoate, and talc have resulted in cosmetic foundation having a SPF value of 25.7. Sericin in sunscreen composition enhances the light screening effect of UV filter like triazines, and cinnamic acids ester[65].

Nail cosmetics, containing 0.02-20 per cent sericin are reported to prevent nail from chapping, brittleness, and imparting the inherent gross to nails[66]. Hair and bath preparations, containing 0.02-2 per cent sericin and 0.01-1 per cent olive oil, fatty acid or their salts show reducing damage of hair surface by binding of sericin to hairs[67]. Sericin hydrolysates with average molecular weight 300-3000 are used as conditioners for skin and hair[68]. Shampoo containing sericin and pelarogenic acid of pH less than six are useful for the care and cleaning of hairs[69].

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