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PURIFICATION AND CHARACTERIZATION OF GLUE LIKE SERICIN PROTEIN FROM A WILD SILKWORM *Antheraea assamensis* Helfer

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ABSTRACT
Sericin is a hot water soluble glycoprotein and has been partially characterized in the domesticated silkworm *Bombyx mori*. Contrary to this very little information is available about the sericin of non mulberry silkworms. Sericin is generally a waste product during degumming. Recent advancement in silkworm research has revealed the use of sericin of *B. mori* as protein based biomaterial involving preparation of sericin 2D and 3D matrices, hydrogels and growth of fibroblast cells on sericin bio- films. The present study discusses the properties of sericin extracted from a wild silkworm *Antheraea assamensis* (Muga Silkworm) confined to the North Eastern Region of India and producing the golden hued muga silk. Molecular weight determination was done by SDS- PAGE. Sericin was characterized using SEM, FT-IR, XRD, and DSC. SDS- PAGE of sericin showed presence of both high and low molecular weight fractions. Surface morphology by SEM showed a rough surface along with aggregation of small particles of size around 1- 2 µm. Structure determination by FT-IR and XRD revealed the presence of both α- helical and β- sheet structures. Thermal properties were studied by DSC showing a degradation temperature around 363^0C. It is expected that this study will be helpful in exploiting sericin as potential biomaterial in biomedical and allied fields.

KEYWORDS: biomaterials, structure, thermal properties, biomedical, SDS- PAGE.

INTRODUCTION

Silk, a mass-producible natural polymer, is mainly produced by Lepidopterean insects of the family Bombycidae and Saturniidae. Silk from domesticated silkworms of the Bombycidae family (*Bombyx mori*) is known as mulberry silk and silk from all other sources are called non- mulberry silks. *Bombyx mori* silk is the most extensively studied silk till date and the non mulberry silk still remain unexplored and requires standardization in every aspect. Silk fibers are composed of two proteins, a fibrous core *i.e* fibroin and a family of adhesive silk proteins called sericin. Fibroin, the major structural protein is secreted from posterior silk gland and sericin is biosynthesized in the middle silk gland of the mature silkworm larvae (Dash *et al*, 2006). Sericin binds the fibroin fibers together in a cocoon (Fedic *et al*, 2002) Sericin is a hot water soluble glycoprotein (Gamo *et al*, 1977) and has been partially characterized in the domesticated silkworm *B. mori*. Contrary to this very little information is available about the sericin of non mulberry silkworms. Ahmed *et al,* 2004 reported a 66kda sericin fraction from the cocoons of *Philosamia ricni* and *Antheraea assamensis.* In *Antheraea mylitta* cocoon peduncle a 200kda sericin has been reported by Dash *et al*, 2006. Sericin is generally a waste product during degumming, i.e removal of sericin by boiling in hot alkaline solution to improve the fiber quality. The focus in recent years has been to obtain further knowledge about the biological properties of sericin and the various advantages of this protein. Advancement in silkworm research has revealed the use of sericin of *B. mori* as protein based biomaterial (Zhang *et al*, 2002, Teramoto *et al*, 2005) involving preparation of sericin 2D and 3D matrices (Mandal *et al*, 2009), hydrogels (Teramoto *et al* , 2005) and growth of fibroblast cells on sericin biofilms (Tsubouchi *et al.,* 2005).

Antheraea assamensis is a wild silkworm confined to the North Eastern Region of India and producing the golden hued muga silk. Being a semi domesticated silkworm, it is normally exposed to harsh environmental stresses like heat and drying. Therefore it is important to study the properties of sercin which may contribute to the improved environmental stability. An attempt has been made to study the structure and properties of sericin of this wild species which may be helpful in exploiting it as potential biomaterial.

MATERIALS AND METHODS

Cocoons of muga silkworm (*Antheraea assamensis* Helfer) were obtained from rearing house of Institute of Advanced Study in Science & Technology (IASST), Assam, India. All fine chemicals and reagents were procured from Sigma Aldrich. Double distilled water (Milipore, USA) was used in the study.

Isolation of Sericin from cocoons

The sericin was isolated from cocoons by the method described by Takasu *et al*. 2002 with a slight modification. In brief, the finely cut peduncle pieces were weighed and soaked in a solution containing 8M urea, 1% SDS and 2% β-mercaptoethanol for 30 min at room temperature and then kept at 80 ◦C for 5 min. After the removal of residual

fiber the supernatant was centrifuged to get sericin solution. The sericin was collected as precipitate by adding three volumes of ethanol followed by storing at −20◦C for an hour. 20mM Tris–HCl was added to dissolve the precipitate. The protein concentration was determined by Lowry protein assay method (Lowry *et al*, 1951). 8% SDS-PAGE was performed (Laemmli, 1970) under reducing condition.

Surface morphology of sericin

Scanning electron microscopy (SEM) images of sericin were obtained after gold sputtering using a JEOL JSM-5800 scanning electron microscope with incident electron beam energy of 15 kV.

Structure determination

FT- IR Analysis

Fourier transform infrared (FTIR) analyses of the samples were carried out using a Bruker, vector 22 FTIR spectrometer. To avoid the effect of moisture the samples were dried overnight in a desiccator. The IR spectra were obtained in the spectral region of 400–4000 cm^{-1}.

XRD Analysis

Wide-angle XRD patterns of the samples were measured by an X-ray diffractometer (PANalytical, X'PertPRO PW3040/60) using CuKα radiation (λ = 1.54 Å) in the 2θ range of 5–40^0 at 40 kV, 40 mA.

Thermal Properties

Differential scanning calorimetry (DSC)

The thermal properties of the samples were measured in a Perkin Elmer, DSC 6000, USA under a dry nitrogen gas flow of 50 ml min^{-1}. The samples were heated at 5^0C min^{-1} from 30^0 to 350^0C.

RESULTS

Isolation and Purification of Sericin

SDS- PAGE of sericin from the cocoons of *A. assamensis* on an 8% gel in reducing condition showed distinct bands with masses of 66 and 100 kDa and a smear in the high molecular weight range (Fig. 1). However, low molecular weight smears were observed in the range of 50- 36 kDa.

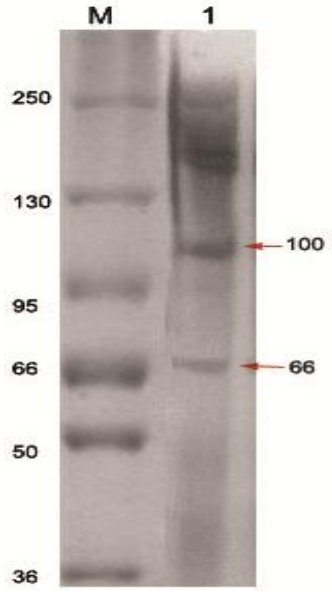

FIGURE 1: 8% SDS- PAGE of sericin from A. *assamensis*. M- Molecular weight marker, 1- sericin protein

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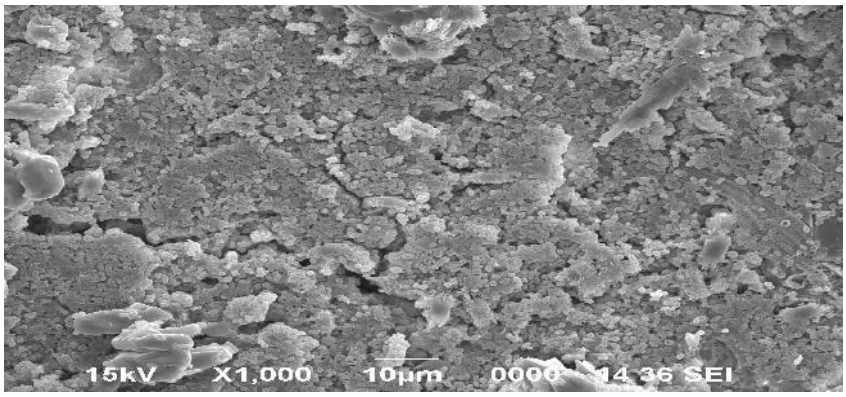

FIGURE 2. SEM images of sericin from *A. assamensis.*

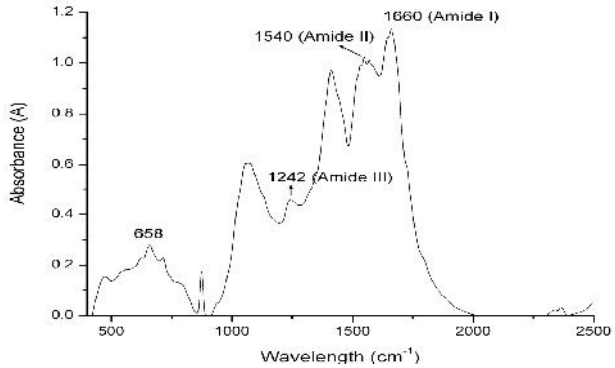

FIGURE 3. FT-IR spectra of sericin from *A. assamensis* in the spectral range of 400- 4000cm^{-1}

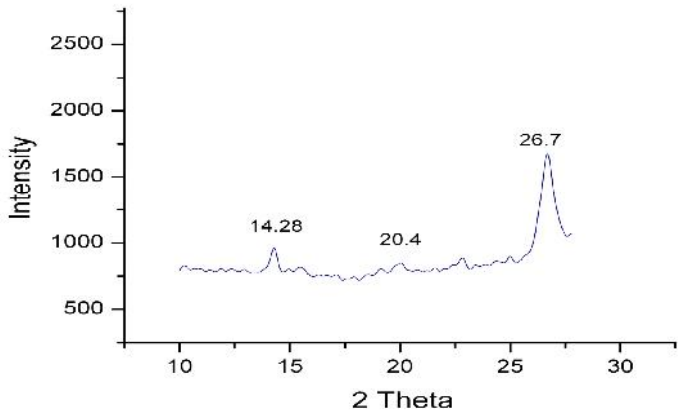

FIGURE 4. XRD spectra of sericin from *A. assamensis*

SEM Studies

Scanning Electron Microscopy images of sericin are shown in (Fig. 2). Small spherical particles in the range of 1- 2 µm were observed. Aggregation of these particles occurred forming an uneven surface.

Structure determination

The secondary structure of sericin was determined by FT-IR and XRD studies. In FT-IR the protein conformation was determined by three distinguishable vibration peaks related to protein amide by identifying the peak positions of amide I, II, and III. The peak positions of amide I (C=O stretching), amide II (N–H deformation and C–N stretching) and amide III (C–N stretching and N–H deformation) of sericin derived from *A. assamensis* were found at 1,660, 1,540 and 1,242 cm^{-1}, respectively (Fig. 3).

The result of X- Ray Diffraction curve intensity is shown in (Fig. 4). Sericin showed diffraction peaks at 2θ angle of 14.2, 20.4, and 26.70.

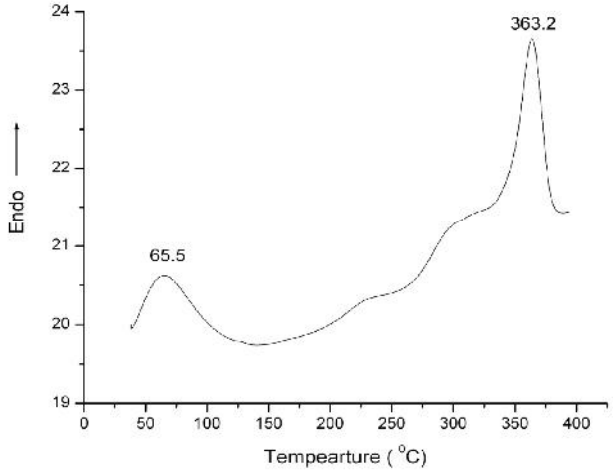

FIGURE 5. DSC spectrum of sericin from *A. assamensis*

Thermal Properties

Differential scanning calorimetry was used to study the thermal properties of sericin (Fig. 5). The first endothermic peak was found around 65.5^0C and the second major peak was around 363.2^0C along with two shoulder peaks at 230 and 300^0C.

DISCUSSION

Sericin was extracted by using urea and precipated by adding ethanol. SDS- PAGE showed a prominent band at 66 kda reported for sericin from *Antheraea assamensis* and *Philosamia ricini* by Ahmed *et al*, 2004. A band at 100kda was also observed which has not been reported elsewhere. A smear was found in the high molecular weight range from 150- 200kda. Dash *et al*, 2007 reported sericin bands of 200kda and more than 200kda in the cocoons of *Antheraea mylitta.* Another smear was found in the region of 50- 36kda which indicates low molecular weight sericin in the cocoons of *A. assamensis*. It has been reported that high molecular weight sericin increases the strength of the fiber while the lower molecular weight sericin protects the pupa from various environmental stresses (Dash *et al*., 2007). Surface topography observed by SEM images of sericin showed a rough surface

morphology. This roughness is assumed to be the result of the alcohol treatment used during the precipitation of the protein by ethanol. The alcohol treatment induces crystallinity that produces a stretching force inducing rough surface occurrence (Dash *et al*. 2009). The size of the sericin particles were found to be in nanoscale range. In FT- IR spectroscopy, Amide I (1,600–1,690 cm−1) absorption primarily represents the C=O stretching vibration of the amide group. The peak at 1665 cm^{-1} for sericin has been assigned to α- helix by Tretinnikov *et al*., 2001 and Teramoto *et al.,* 2005. Amide II (14,80–1,575 cm−1) absorption contains contributions from N−H bending and C−N stretching vibrations; in this case the amide II peak was evident around 1540 cm^{-1} indicating random coil and β turn conformations (Nayak *et al*., 2012). The amide III (1,229–1,301 cm−1) arises mainly from the C−N stretching vibration coupled to the N−H in-plane bending vibration. Minzong Li *et al*, 2003 has assigned the 1242 cm^{-1} band to α- helical conformations. Therefore, sericin from *A. assamensis* is found to be amorphous with α- helical and random coil conformations with a negligible amount of β conformations.

Secondary structure was further studied by XRD. Sericin usually shows two diffraction peaks, a minor peak around $2\theta = 19.2^0$ and a comparatively major peak 23.2^0 (Nagura *et al*., 2001; Tao *et al*., 2005). However in case of sericin from *A. assamensis* major peak were evident around 20.4^0 and 26.7^0 along with a peak at 14.2^0. Miyake *et al*. (2003) studying high molecular weight sericin films of *Bombyx mori* found a diffraction peak near $2\theta = 20^o$, and a shoulder peak at near $2\theta = 12^o$, 28^o, and 43^o. Earlier studies suggested native sericin containing both random coils and β-sheets representing amorphous and crystalline regions respectively (Dash *et al*., 2007; Tsukada *et al*., 1981; Teramoto *et al*., 2006) which may be considered similar in case of sericin from *A. assamensis* under study.

The most convenient method of studying the thermal properties of macromolecules is Differential scanning calorimeter. In the case of sericin from *A. assamensis*, an endothermic peak near 65.5°C and another near 363.2°C were observed. The former peak signifies the molecular mobility and melting induced thermally, while the latter indicates thermal decomposition (Mandal *et al*. 2011). The observed degradation temperature corresponded to an endothermic peak of sericin powder of A. mylitta near 141°C and at 311°C that were reported earlier (Mandal *et al*. 2011). Endothermic degradation temperatures of sericin powder obtained from *Bombyx mori* at high-temperature and high-pressure degumming were also reported to be at 210°C (Aramwit *et al*., 2010a). The thermal stability of sericin is influenced by the use of chemicals during the extraction process (Lamoolphak et al., 2008). The higher degradation temperature of sericin from *A. assamensis* compared to *A. mylitta* and *Bombyx mori* indicates higher thermal stability.

CONCLUSION

This study, aims to understand the properties of sericin from *A. assamensis* and its possible applications in the biomedical field. SDS- PAGE of sericin obtained by urea extraction showed a range of molecular weights which may have a protective role in pupal growth and to overcome harsh environmental stress, as this silkworm is wild in nature. Electron microscopy images showed the surfaces to be rough and the secondary structure was found mainly amorphous with little amount of crystalline structures. The result of the thermal property was interesting, as it showed high degradation temperature of sericin compared to others. This thermal stability may be an added advantage to exploit it as a potential biomaterial.

REFERENCES

Ahmad, S.E., Kamra, A., and Hasnain, S.E. (2004) Fibroin silk proteins from the nonmulberry silkworm *Philosamia ricini* are biochemically and immunochemically distinct from those of mulberry silkworm *Bombyx mori*. *DNA Cell Biol.* 23, 149–154.

Aramwit, P., Kanokpanont, S., Nakpheng, T., Srichana, T. (2010a) The effect of sericin from various extraction methods on cell viability and collagen production. Int J Mol Sci. 11, 2200–2211

Dash, B.C., Mandal, B.B., and Kundu, S.C. (2009) Silk gland sericin protein membranes: fabrication and characterization for potential biotechnological applications. *J Biotechnol*. 144, 321–329.

Dash, R., Mukherjee, S., and Kundu, S.C. (2006) Purification and biochemical characterization of a 70 kDa sericin from tropical tasar silkworm, *Antheraea mylitta*. *Int J Biol Macromol.* 38, 255– 258.

Dash, R., Ghosh, S.K., Kaplan, D.L., and Kundu, S.C. (2007) Purification and biochemical characterization of a 70-kDa sericin from tropical tasar silkworm, *Antheraea mylitta*. *Comp. Biochem. Physiol*. B. 147, 129- 134.

Fedic, R., Zurovec, M., Sehnal, F. (2002) The silk of Lepidoptera. *J. Insect Biotech. Sericol*. ,71, 1- 15.

Gamo, T., Inokuchi, T., Laufer, H. (1977) Polypeptides of fibroin and sericin secreted from the different sections of the silk gland in *Bombyx mori*. Insect Biochem. 7, 285–295.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature* 227, 680–685.

Lamoolphak, W., De-Eknamkul, W., Shotipruk, A. (2008) Hydrothermal production and characterization of protein and amino acids from silk waste. Bioresour Technol. 99, 7678–7685

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.

Mandal, B. B., Priya, A. S., and Kundu, S.C. (2009) Novel silk sericin/gelatin 3-D scaffolds and 2-D films:

Fabrication and characterization for potential tissue engineering applications. *Acta Biomater*. 5, 3007–3020.

Mandal, B. B., Ghosh, B., and Kundu, S.C. (2011) Non-mulberry silk sericin/ poly (vinyl alcohol) hydrogel matrices for potential biotechnological applications. *Int J Biol Macromol*., 49(2), 125–133

Mingzhong, L., Wei, T., Shenzhou, L., Shigenori, K. (2003) Compliant film of regenerated Antheraea pernyi silk fibroin by chemical crosslinking. *Int. J. Biol. Macro*. 32, 159- 163.

Miyake, H., Wakisaka, H., Yamashita, Y., Nagura, M. (2003) Moisture characteristic and structure of high molecular weight sericin films. *Polymer Journal*. 35(8), 683- 687

Nagura, M., Ohnishi, R., Gitoh, Y., Ohkoshi, Y. (2001) Structure and physical properties of cross-linked sericin membranes. *J Sericultural Sci Jpn* 70, 149–153

Nayak, S., Talukdar, S., and Kundu, S. C. (2012) Potential of 2D cross linked sericin membranes with improved biostability for skin tissue engineering. *Cell Tissue Res.,* 347, 783–794

Tao, W., Li, M., Xie, R. (2005) Preparation and structure of porous silk sericin materials. Macromol Mater Eng 290, 188–194

Teramoto, H., Kakazu, A., Asakura, T. (2006) Native structure and degradation pattern of silk sericin studied by 13C NMR spectroscopy. *Macromolecules*, 39, 6–8.

Teramoto, H., Miyazawa, M. (2005) Molecular orientation behavior of silk sericin film as revealed by ATR infrared spectroscopy. *Biomacromolecules* 6, 2049–2057.

Teramoto, H., Nakajima, K., Takabayashi, C. (2005) Preparation of elastic silk sericin hydrogel. Biosci. Biotechnol. Biochem. 69, 845–847.

Tsubouchi, K., Igarashi, Y., Takasu, Y., Yamada, H. (2005) Sericin enhances attachment of cultured human skin fibroblasts. *Biosci. Biotechnol. Biochem*. 69, 403–405.

Tsukada, M., Bertholon, G. (1981) Preliminary study of the physicochemical characteristics of silk sericin. *Bull. Sci. Inst. Text. Fr*. 10, 141–154.

Takasu, Y., Yamada, H., Tsubouchi, K. (2002) Isolation of three main sericin components from cocoon of the silkworm, *Bombyx mori*. *Biosci. Biotechnol. Biochem*. 66, 2715–2718.

Zhang, Y.Q., (2002) Applications of natural silk protein sericin in biomaterials. *Biotechnol. Adv*. 20, 91–100.