Abstract

Antheraea mylitta is an economically important silkworm and grown in the Eastern part of India. Two soluble proteins, arylphorin and sericin, are produced in the silk gland of these silkworms and are essential for insect growth and development. The arylphorin protein was extracted from the silk gland, purified and identified by N-terminal amino acid sequencing. MALDI-TOF analysis showed similarity with *Antheraea pernyi* arylphorin. Based on the N-terminal and C-terminal regions of *A. pernyi* arylphorin, primers were designed, and *A. mylitta* arylphorin cDNA was cloned and sequenced. The complete cDNA (2215 bp) includes 5' UTR of 25 bp, an ORF of 2115 bp which could encode a protein of 704 amino acids having molecular weight 83 kDa and 3' UTR of 75 bp. Homology modelling was done using *A. pernyi* arylphorin as a template. The ORF of arylphorin cDNA was expressed in *E. coli* as insoluble and in *Drosophila* S2 cells as soluble 83 kDa His-tagged fusion protein and purified through Ni-NTA chromatography. Analysis of tissue-specific expression of arylphorin by realtime PCR showed maximum expression in the fat body followed by silk gland and integument. 5' flanking region (759 bp) of arylphorin gene was amplified by inverse PCR and the full-length gene (5359 nucleotides) containing five exons and four introns was cloned from the *A. mylitta* genomic DNA and sequenced. A polyclonal antibody was raised against purified arylphorin and more native arylphorin protein (500 kDa) was purified from the fat body. Study of mitogenic effect of native, recombinant and chymotrypsin hydrolysate of arylphorin on different insect cell lines showed that arylphorin could be used as a serum substitute to grow insect cell line in vitro.

Crude silk sericin protein (10-300 kDa) was isolated from *Antheraea mylitta* cocoon, fractionated into three different molecular size-ranges fractions such as fraction-1 (50-300 kDa), fraction-2 (30-50 kDa) and fraction-3 (10-30 kDa), and used to prepare crude sericin nanoparticles (CRSNPs), as well as fraction specific nanoparticles: n-SNP1, n-SNP2, and n-SNP3, respectively having negative surface potential. Poly-L-lysine coating was done to make the surface potential positive (p-SNPs) and confirmed through UV-Vis spectroscopy, FTIR, zeta sizer and zeta potential measurement. The shape and sizes of all SNPs were determined by electron microscopy and found to be spherical having a diameter ranging from 115-165 nm

(CRSNPs), 69-85 nm (SNP1), 33-49 nm (SNP2) and 14-24 nm (SNP3) for n-SNPs and p-SNPs, respectively. Evaluation of the antibacterial activity of all these SNPs showed significantly more activity of p-SNPs than n-SNPs against *Staphylococcus aureus* and *Escherichia coli*. Among all SNPs, SNP2 showed the strongest antibacterial activity followed by SNP3, SNP1, and CRSNPs. Relatively higher reactive oxygen species (ROS) generation was observed after the treatment of bacteria with p-SNP2 (50 μg/mL) which is non-toxic to animal cells. FE-SEM analysis showed a relatively higher disruption of bacterial cell membranes after treatment with p-SNPs than n-SNPs. Furthermore, pSNPs (specifically pSNP2) showed significantly more antioxidant activities than n-SNPs in ABTS and DPPH free radicals scavenging assay, as well as protective effects against hydroxyl radical-induced oxidative DNA damage and lipid peroxidation inhibition. Moreover, p-SNP3 showed potent anti-proliferative activity than any other SNPs against breast cancer cells (MDA-MB 231), arresting cell cycle in the G1 phase without affecting the growth of normal keratinocyte (HaCaT) cells. These results suggested that anti-bacterial, antioxidant and anti-proliferative activities of SNPs depend on molecular size and surface potential.

One of the sericin proteins (150 kDa) was extracted from the white silk fibre (WSF) of the matured tasar silkworm larvae, purified by size exclusion chromatography and characterized by 2D-gel electrophoresis, FT-IR and CD spectroscopy. Based on the amino acid sequences (NH_2–GSGTDEDSDD–COOH) (showed similarity with *B. mori* sericin 3 gene) of the purified protein (determined by MALDI-TOF mass spectrometry) primer was designed, a 570 bp DNA band was amplified by PCR using silk gland cDNA as template. The nucleotide sequence showed similarity (when searched by BLAST) with a hypothetical protein of *Lonomia obliqua* whose functions were found similar like sericin protein. The multiple sequence alignment of this cDNA sequence shows about 39% identity with *B. mori* sericin 3 cDNA and 56% similarity with *B. mori* sericin 3 amino acid sequences. These results indicate partial cloning of cDNA encoding 150 kDa putative sericin protein of *A. mylitta*.

Keywords: *Antheraea mylitta*, arylphorin, sericin, sericin nanoparticle, molecular cloning and sequencing.