Abstract

The fibroin protein isolated from the posterior silkgland of tropical saturniid silkworm *Antheraea mylitta*, was solubilized in lithium dodecyl sulfate and purified by gel filtration. The major fraction from gel filtration was analyzed by SDS-polyacrylamide gel electrophoresis under non-reducing and reducing conditions. One major protein band of approximately 395 kDa was obtained under non-reducing condition and a doublet band of ~197 kDa under reducing condition. The appearance of a single spot in two-dimensional electrophoresis confirmed the purity of the protein indicating that it maybe a homodimeric protein of two similar sized polypeptides. Amino acid composition analysis showed that, like other saturniid fibroins, it is rich in glycine, alanine and serine amino acids. N-terminal amino acid sequence shows significant homology with other *Antheraea* species. The enzymatic deglycosylation analysis indicates that the fibroin protein is glycosylated and the oligosaccharides are O-linked to protein backbone by N-acetylgalactoseamine moiety. Northern blot hybridization of the silkgland total RNA from the larval developmental stages using fibroin gene as probe showed that fibroin is expressed in the intermoult stages and repressed during the moulting stages, reaching a peak in the fifth intermoult. Immunoblot analysis of fibroin protein production with the raised anti-fibroin antibody confirmed the differential fibroin expression, in accordance with fibroin mRNA synthesis. Dot blot hybridization of genomic DNA isolated from each larval developmental stage showed that fibroin gene amplification did not occur at any stage of larval development. Fibroin gene expression in *A. mylitta*, like in *B. mori*, is thus transcriptionally controlled and shows differential temporal variations. Genomic amplification of the partial fibroin gene by PCR revealed that the nucleotide sequence of the exon 1 and exon-intron boundaries are highly conserved among other *Antheraea* and *Bombyx* species. However the exon 2 regions of *Antheraea* and *Bombyx* species are unrelated in that the former contains several polyalanine stretches while the latter shows GX (G, glycine; X, alanine/ serine/ tyrosine/ valine/ threonine) di-peptides. The 5′ flanking region of the fibroin gene housing the putative TATA box region was ascertained to be the functional promoter of the gene by promoter assay with GFP (Green Fluorescent Protein) as reporter gene. An attempt to clone the entire fibroin gene was undertaken by construction of a genomic DNA library, screening the library with fibroin-specific probe and complete nucleotide sequencing of the recombinant lambda DNA from the screened clone. The nucleotide sequence of 12715 nucleotides revealed that the fibroin gene of *A. mylitta* is truncated by a sandwich structure consisting of a central 8387 nucleotides of complete Pao-like LTR retroelement, rtAmy (retrotransposon ***A. mylitta***) flanked on its either sides by 1438 nucleotides of direct repeats. The disruption of the fibroin gene by the sandwich structure may attribute to produce the 'naked pupa' mutants that have been found to be fibroin secretion deficient in *B. mori*.

Keywords: Saturniid silkworm; fibroin; *Antheraea mylitta*; O-glycosylation, differential expression; green fluorescent protein; genomic DNA library; Pao-retrotransposon.