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Effect of a *Bombyx mori* Protein Disulfide Isomerase on Recombinant Protein Secretion

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Insect baculovirus expression vector system is extensively applied to synthesize many kinds of heterologous important proteins for diagnostics, therapeutics, structural and functional studies. The system has proved particularly useful for producing recombinant proteins because those proteins are authentically folded, proteolytically processed, post-translationally modified, and biologically active. However, these recombinant proteins sometimes forms insoluble aggregates and their biological activity was reduced. The formation of these aggregates suggests a limitation in folding, assembly, and secretion pathway. Recently, specific enzymes that catalyze or molecular chaperone protein folding and other secretory process have been discovered, and these are limited in the insect expression system.

To obtain molecular chaperone and ER foldase related genes from *Bombyx mori* Bm5 cell lines, the cDNA library was constructed from tunicamycin treated BM5 cell lines. Tunicamycin inhibits *N*-glycosylation. Forthy differentially expressed clones were isolated and we focused TmInc131 clone which has high similarity with protein disulfide isomerase gene from *D. melanogaster*. I named TmInc131 as "bPDI" (*Bombyx mori* protein disulfide isomerase).

A bPDI cDNA encodes the 494 amino acids and its molecular weight is 55.6 kDa. The bPDI protein contains two conserved thioredoxin catalytic domains and ER retention signal, KDEL at its C-terminals. No putative glycosylation site was detected in the bPDI protein sequence.

The expression of bPDI mRNA was up-regulated during ER stress by calcium ionophore A23187, tunicamycin, or dithiothreitol. They induce an accumulation of unfolded proteins in ER. bPDI mRNA is expressed predominantly in the fat body of insects. Hormonal regulation studies showed that juvenile hormone,

insulin, and a combination of juvenile hormone and transferrin affected bPDI mRNA expression. A challenge with exogenous bacteria also affected expression with the peak at 16 hours after infection. These results suggest that bPDI, a member of the ER-stress protein group, may play an important role in exogenous bacterial infection of the fat body, and that its expression is regulated by hormones.

To assay the enzymatic activity, bPDI was expressed in Sf9 cells using the baculovirus expression vector system. The bPDI recombinant proteins were purified and tested for PDI activity by the reduced and scrambled RNase folding method. While the spontaneous refolding of RNase was very slow, the presence of recombinant bPDI and bovine liver PDI accelerated RNase folding. It indicates that bPDI also has disulfide isomerase activity.

Recent studies have demonstrated that misfolded proteins are accumulated in many diseases including Alzheimer's, goiter, emphysema, and prion infections. bPDI was over-expressed or knock-downed in Sf9 cells to study the relationship between bPDI expression and protections against protein misfolding. bPDI gene was cloned in insect expression vector pIZT/V5-His for over-expression and bPDI double-stranded RNA (dsRNA) was generated for knock-down. Over-expression of bPDI significantly improved survival rate, but bPDI dsRNA transfection significantly reduced survival rate after 48-hr exposure. In mock-transfected or wild-type cells had no significant effect. This results support the view that bPDI is an important component of defence mechanism against protein misfolding.

To study the relationship between bPDI expression and silk synthesis in *Bombyx mori*, bPDI dsRNA was generated. After bPDI dsRNA injection into the 1st-5th instar larvae, naked pupa and sericin cocoon (containing only sericin but no fibroin) were observed. These results supports the view that bPDI is also an important component of silk synthesis in *Bombyx mori*.

The insect baculovirus expression vector system is useful for the production of biologically active recombinant proteins. However, over-expression of foreign proteins in this system often make protein aggregates due to the translated protein folding problem. In order to overcome this limitation in insect baculovirus expression vector system, I developed a versatile baculovirus expression and secretion system using bPDI as a gene fusion partner. The production of antibacterial peptide fusion proteins with bPDI improved the peptide production by 20 times. This modified

and improved baculovirus expression vector system expression with bPDI gene fusion will be very useful for the production of biologically active recombinant proteins.