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Effect of a *Bombyx mori* PDI on Recombinant Protein Secretion

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Protein disulfide isomerase (PDI) found in the endoplasmic reticulum (ER) catalyzes disulfide bond exchange andassists in protein folding of newly synthesized proteins. PDI also functions as a molecular chaperone and has been found to be associated with proteins in the ER. In addition, PDI functions as a subunit of two more complex enzyme systems: the prolyl-4-hydroxylase and the triacylglycerol transfer proteins. AcDNA that encodes protein disulfide isomerase was isolated from *Bombyx mori* (bPDI), in which an open reading frame of 494 amino acid (55.6 kDa) is shown. In addition, PDI-typical two thio-redoxin active sites of CGHC and an ER retention signal of KDEL motif are shown at its C-terminal. The bPDI protein shared less than 55% of the amino acid sequence homology with other reported PDIs. The bPDI is most genetically similar to the *D. melanogaster* PDI (dPDI). Although bPDI shows a relatively low amino acid homology with other PDIs, in which both sites of the two thioredoxin active sites and the ER retention signal are completely conserved. This bPDI cDNA is expressed in insect Sf9 cells as a recombinant proteins using baculovirus expression vector system. The bPDI recombinant proteins are successfully recognized by anti-rat PDI antibody, and shown to be biologically active *in vitro* by mediating the oxidative refolding of reduced and scrambled RNase. On other hand, it has been characterized under ER stress conditions (dominantly induced by calcium ionophore A23187, tunicamycin and DTT), which is known to cause an accumulation of unfolded proteins in the ER. Furthermore, It has also been examined for tissue distribution (pronounced at the fat body and the effect of exogenous bacterial

(peak at 16 h after infection) on the bPDI mRNA expression. Especially, the fact that the expression time of bPDI in *B. mori* infected with bacteria was coincide with that of anti-bacterial proteins suggested that bPDI is related with insect immune response. To examine the relation between bPDI and immune, we established PDI-transformed cell lines using pIZT-V5/HIS and bPDI knock-out insect cell lines by RNA interference (RNAi) method. And then, following the treatment of 10 mM DTTon both cells, the survival rate of cell was investiged. As the result, the survival rate of cell overexpressing bPDI is remarkably high (17%) compared with normal cell (14%), whereas that of the bPDI knock-out cell is considerably low (2%) with normal cell. Therefore, we suggest that bPDI is involved in immune response.

Increasing PDI activity in bacterial, yeast and mammalian cell expression systems can lead to increased secretion of heterologous protein containing disulfide bridges. Since insect cells are widely used for the expression of recombinant proteins. We have developed secretion system based on the use of bPDI as a gene fusion partner. Fusion with bPDI increased the extracellular production of heterologus proteins (nuecin, 20-fold; enbocin, 14-fold; nuecin+enbocin; 20-fold). Linkage to bPDI prevented the aggregation of the secreted proteins, resulting in high-level accumulation of fusion protein in soluble and biologically active forms. As the above results, we suggest that bPDI may play an important role in protein folding mechanism of insects.