2-17. A Novel Cellulase of the Mulberry Longicorn Beetle, *Apriona germari*, Dependent on *N*-Glycosylation for Enzymatic Activity

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A novel -1,4-endoglucanase (EGase, EC 3.2.1.4) cDNA belonging to glycoside hydrolase family (GHF) 45 was cloned from the mulberry longicorn beetle, *Apriona germari*. The cDNA encoding EGase of *A. germari* (Ag-EGase) is 711 base pairs long with an open reading frame of 237 amino acid residues. The deduced protein sequence of Ag-EGase showed 54% and 48% identity to phytophagous beetle *Phaedon cochleariae* and termite *Reticulitermes speratus* hindgut symbiont, respectively. The putative catalytic sites of GHF 45 are conserved in Ag-EGase. Southern blot analysis of genomic DNA suggested the presence of Ag-EGase gene as a single copy and Northern blot analysis confirmed midgut-specific expression at transcriptional level. Similarly, the Ag-EGase enzyme assay exhibited high activity in only midgut tissue, suggesting the midgut is the site where large quantities of EGase are synthesized for degrading the absorbed cellulose from the diet. The cDNA encoding Ag-EGase was expressed as a 29-kDa polypeptide in baculovirus-infected insect Sf9 cells

and culture supernatants of the recombinant baculovirus-infected cells showed EGase enzyme activity of 15.25 U per ml of medium containing 0.5 106 cells at 5 days postinfection. The enzymatic properties of purified recombinant Ag-EGase expressed in baculovirus-infected insect cells showed that the Ag-EGase activity was most effective at 50 °C and pH 6.0, and was stable at 55 °C for at least 10 min. Furthermore, *N*-glycosylation of the recombinant Ag-EGase was revealed by tunicamycin to the recombinant virus-infected Sf9 cells and by endoglycosidase F to the purified recombinant Ag-EGase, demonstrating that the carbohydrate moieties are not necessary for secretion but essential for Ag-EGase enzyme activity.