

## **The Role of N-Glycosylation of a Beetle (*Apriona germari*) Cellulase That Is Necessary for Enzyme Activity**

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We have cloned a cellulase [ $\beta$ -1,4-endoglucanase (EGase), EC 3.2.1.4] cDNA (Ag-EGase I) belonging to glycoside hydrolase family (GHF) 45 from the mulberry longicorn beetle, *Apriona germari*. The Ag-EGase I gene spans 2713 bp and consisted of two introns and three exons coding for 237 amino acid residues. The catalytic sites of GHF 45 are conserved in Ag-EGase I and has a potential N-glycosylation site at Asn97. We first describe here the N-glycosylation and its role for enzymatic activity in EGase of insect origin. The N-glycosylation of Ag-EGase I was revealed by tunicamycin to the recombinant virus-infected insect Sf9 cells and by endoglycosidase F to the purified recombinant Ag-EGase I, demonstrating that the carbohydrate moieties are not necessary for secretion but essential for Ag-EGase I enzyme activity. To elucidate further functional role of the N-glycosylation in Ag-EGase I, we have assayed the cellulase enzyme activity in Asn97Gln mutant. Lack of N-glycosylation at site 97 showed no substantial enzyme activity. This result demonstrates that N-glycosylation is necessary for enzyme activity, with N-glycosylation at site 97 playing the essential role.