

## N-Glycosylation of a Beetle (*Apriona germari*) Cellulase Ag-EGase II Is Necessary for Enzymatic Activity

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We previously reported that the  $\beta$ -1,4-endoglucanase (EGase) belonging to glycoside hydrolase family (GHF) 45 cloned from the mulberry longicorn beetle, *Apriona germari* (Ag-EGase II) has three potential N-glycosylation sites at 56-58 (NKS), 99-101 (NST), and 237-239 (NYS) amino acid residues. In the present study, we have analyzed the functional role of these potential N-glycosylation sites. Tunicamycin treatment completely abolished Ag-EGase II enzyme activity. To further elucidate the functional role of the N-glycosylation sites in Ag-EGase II, we have assayed the cellulase enzyme activity in Ser58Gln, Thr101Gln, or Ser239Gln mutants. Lack of N-glycosylation at site 99-101 (NST), which is conserved in known beetle GHF 45 cellulases, showed no substantial enzyme activity and reduced the molecular mass. However, the mutations in Ser58Gln or Ser239Gln did not affect the enzyme activity and the shift in molecular mass. The present study demonstrates that N-glycosylation at site 99-101 amino acid residues (NST) is not essential for secretion but necessary for Ag-EGase II enzyme activity.