

Suppression of β -*N*-acetylglucosaminidase in
N-glycosylation Pathway for Complex Glycoprotein Formation in *Drosophila* S2 Cells

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Most of insect cells have a simple *N*-glycosylation process and consequently, paucimannosidic or simple core glycans are predominant. Previously, we also revealed that paucimannosidic *N*-glycan structures are dominant in *Drosophila* S2 cells. It has been proposed that GlcNAcase, a hexosaminidase that exists in Golgi membrane and cuts off a terminal GlcNAc, might be a factor for simple *N*-glycosylation in several insects and their derived cells. In the present work, we investigated substantial suppression effects of GlcNAcase on *N*-glycan patterns in *Drosophila* S2 cells using two suppression strategies; RNAi that is a mRNA-level method and specific chemical inhibitor, 2-ADN, that is a protein-level method. Compared to the original *N*-glycan sample from hEPO-secreting stably transfected S2 cells, we found that improved *N*-glycan structures were clearly shown to have a terminal GlcNAc and/or galactose through HPLC and MALDI-TOF MS analyses. Therefore, we proved that GlcNAcase is a possible major factor for formation of paucimannosidic core *N*-glycans in *Drosophila* S2 cells. These data suggest that complex glycoproteins might be possible in the engineered *Drosophila* S2 cells by suppression of GlcNAcase in *N*-glycosylation pathway.