Analysis of the Asparagine-linked Oligosaccharides of Recombinant Human Glycoproteins Produced in the Porcine Mammary Gland

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Glycosylation of recombinant proteins is of particular importance because it can play significant roles in the clinical properties of the glycoprotein, such as enzyme activity, protein stability, pharmacokinetics, and immunogenicity. In this work, we determined the structure of the N-glycans of recombinant human Factor IX and Protein C produced in the transgenic pig mammary gland. Using a combination of capillary electrophoresis, HPLC, and Electrospray Ionization Ion Trap Mass Spectrometry (ESI-IonTrap MS), we have found that the majority of N-glycans of transgenic pig-derived Factor IX and Protein C are complex bi- and tri-antennary with one or two terminal N-acetylneuraminic acid (Neu5Ac) moieties (over 95%). Using these highly sensitive techniques, we were unable to detect any high mannose or hybrid N-glycan structures. We also found that the N-glycan structures of the glycoproteins produced in the porcine mammary epithelial cells differed with respect to N-glycans from endogenous glycoproteins produced in porcine thyroid, B-cells, and cell-surface glycoproteins. The sialic acid found in transgenic pig derived Factor IX and Protein C was Neu5Ac; we were unable to detect Neu5Gc moieties that are commonly found in porcine glycoproteins that are produced in other tissues and cell types. Additionally, we

were unable to detect any glycans that had a terminal Galα(1,3)Gal disaccharide sequence, which is strongly antigenic in humans. These data indicate that there may be significant tissue/cell-specific differences in N-glycan structures on different porcine tissues. We also have found that N-glycan profiles of tg-FIX are consistent during lactation with respect to the overall distribution of sialylated vs. neutral oligosaccharides. Although the proportion of monosialylated and disialylated structures changed during lactation, the total sialylated structures remained relatively constant at over 95% out of total population of N-glycans. Neutral oligosaccharides, including high mannose structures, were not detected during lactation in measurable quantities by fluorescent HPLC, nor by direct MS analysis. The results show that the porcine mammary gland can be a viable candidate bioreactor for production of recombinant human glycoproteins that require complex, sialylated N-linked glycans.