# Recombinant Mucins with Tailored Glycosylation as Bacterial Toxin Inhibitors

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs Universitet kommer att offentligen försvaras i hörsal Jubileumsaulan, Gula stråket 2B, Göteborg, onsdagen den 16 december 2015, kl. 9.00

av

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Avhandlingen baseras på följande arbeten:

**I.** Maria Cherian, R., Gaunitz, S., Nilsson, A., Liu, J., Karlsson, N.G., and Holgersson, J. Shiga-like toxin binds with high avidity to multivalent O-linked blood group P1 determinants on mucin-type fusion proteins. *Glycobiology* 2014; 24, 26-38.

**II.** Liu, J., Jin, C., Maria Cherian, R., Karlsson, N.G., and Holgersson, J. O-glycan repertoires on a mucin-type reporter protein expressed in CHO cell pools transiently transfected with O-glycan core enzyme cDNAs. *Journal of Biotechnology* 2015; 199, 77-89.

**III.** Maria Cherian, R., Jin, C., Liu, J., Karlsson, N.G., and Holgersson, J. A panel of recombinant mucins carrying a repertoire of sialylated O-glycans based on different core chains for studies of glycan binding proteins. *Biomolecules* 2015; 5, 1810-1831.

**IV.** Maria Cherian, R., Jin, C., Liu, J., Karlsson, N.G., and Holgersson, J. Recombinant mucin-type fusion proteins with Galα1,3Gal substitution as *C. difficile* toxin A inhibitors. *Manuscript*

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# Recombinant Mucins with Tailored Glycosylation as Bacterial Toxin Inhibitors

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## ABSTRACT

Multivalent carbohydrate-based ligands that can inhibit biomedically important protein–carbohydrate interactions have therapeutic potential. One of the important targets for therapeutic intervention is the binding processes mediated through the interactions of bacterial toxins with cell-surface receptors. Inhibition of these interactions has the potential to prevent the toxins from reaching their site of action, and thus, averting the subsequent toxin effects. Even though, multivalent inhibitors that engage in multiple weak interactions can enhance the overall binding interaction, it has been observed that tailoring of specific ligands based on the functional carbohydrate receptor can greatly improve the binding strength of inhibitors.

In this thesis, we have engineered the CHO cell line to produce the recombinant mucin-type fusion protein with tailored glycosylation by expressing P-selectin glycoprotein ligand-1/mouse immunoglobulin G2b (PSGL-1/mIgG2b) together with glycosyltransferases that are known to mediate the biosynthesis of specific carbohydrate determinants. PSGL-1/mIgG2b, which we have proposed as a versatile inhibitor of protein–carbohydrate interactions, consist of the extracellular part of P-selectin glycoprotein ligand-1(PSGL-1) fused to the Fc part of mouse IgG2b. The high density expression of *O*-linked glycans in the mucin part of PSGL-1/mIgG2b provides the scaffold for multivalent display of bioactive carbohydrate determinants, making it suitable as an inhibitor of carbohydrate-binding bacterial toxins, microbial adhesins, viral surface proteins, and antibodies.

In paper I and IV, genetically engineered CHO cells were used to produce PSGL-1/mIgG2b carrying the functional carbohydrate receptors of Shiga toxin 1 and 2 (Stx1 and Stx2) and *C. difficile* toxin A, respectively. The blood group P1 determinant generated in multiple copies on PSGL-1/mIgG2b by the expression of pigeon α4Gal-T and the core 2 enzyme (C2β6GnT-I) bound with high avidity to both Stx1 and Stx2. In Paper IV, PSGL-1/mIgG2b expressing terminal Galα1,3Gal was shown to bind *C. difficile* toxin A and to inhibit its cytotoxic and hemagglutinating properties.

In paper II and III, PSGL-1/mIgG2b was used as a probe to understand the *O*-glycan biosynthesis pathways in CHO cells. The expression of various *O*-glycan core chain glycosyltransferases aided in defining their *in vivo* glycan specificities and their potential competition with the endogenous CHO glycosylation machinery. In paper II, small-scale transient transfections were employed to analyze the effects of *O*-glycan core enzymes, ST6GAL1 and CHST4 on the *O*-glycome repertoire of PSGL-1/mIgG2b. Using these data, in paper III, a panel of recombinant mucins carrying terminal α2,3- or α2,6-linked sialic acid on defined *O*-glycan core saccharide chains was produced by generating stable CHO cell lines. Owing to the pathobiological significance of sialylated glycans, these recombinant mucins will be an important tool for determining the fine *O*-glycan binding specificity of sialic acid-specific microbial adhesins and lectins.

In conclusion, we have recreated the enzymatic pathways involved in the biosynthesis of specific target carbohydrate determinants on defined *O*-glycan chains in CHO cells. Using a mucin-type scaffold has allowed us to create high affinity, multivalent carbohydrate ligands and inhibitors of bacterial toxins.

**Keywords**: *O*-glycans, mucin, bacterial toxin
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