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Studies on *O*-glycosylation of Mucin-Type Proteins and Their Binding to Antibodies, Bacterial Toxins and Viral Receptors

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av
Stefan Gaunitz
M. Sc.

Huvudhandledare:
Prof. Jan Holgersson
Göteborgs Universitet, Inst. för Biomedicin,
Avd. för klin kemi och transfusionsmedicin

Bihandledare:
Ph.D. Anki Nilsson
Recopharma AB

Fakultetsopponent:
Prof. Peter Påhlsson
Linköpings Universitet
Inst. för Klinisk och experimentell medicin,
Avd. för cellbiologi

Betygsnämnd:
Prof. Marion Kusche-Gullberg
Universitetet i Bergen
Inst. for Biomedisin, Matrix Biologi

Prof. Andrej Weintraub
Karolinska Institutet
Inst. för Laboratoriemedicin, Avd. för klinisk mikrobiologi

Prof. Sigvard Olofsson
Göteborgs Universitet
Inst. för Biomedicin, Avd. för infektionssjukdomar

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ABSTRACT

Carbohydrates are ubiquitous on the surface of all cells in mammals where they are involved in interactions with the surroundings (extracellular matrix), other cells (including self and non-self) and microbes (bacteria and virus). Carbohydrate-protein interactions in nature are often mediated via multivalent binding where the combined strength of multiple receptor-ligand interactions results in a binding that is highly specific and strong.

In this thesis we have produced proteins that are densely decorated with carbohydrate determinants in order to study the glycosylation capacity of cell lines (paper I) and generate efficient binders of antibodies (paper II), bacterial toxins (paper III) and virus receptors such as the influenza hemagglutinin (paper IV).

P-selectin glycoprotein ligand-1 (PSGL-1) is a mucin-type protein that is heavily substituted with *O*-glycans. PSGL-1 genetically fused to mouse IgG_{2b} Fc forms a dimeric PSGL-1/mIgG_{2b} mucin-type fusion protein.

In paper I, PSGL-1/mIgG_{2b} was produced in Sf9 (*Spodoptera frugiperda*) and Hi-5 (*Trichoplusia ni*) cell lines. The mucin-type protein was used as a probe to analyze the *O*-glycosylation capacity of these cell lines, which today are used for the commercial production of recombinant proteins and vaccine components. Liquid chromatography-mass spectrometry (LC-MS) revealed that the *O*-glycosylation was more abundant and complex than previously reported which may limit their use for the production of therapeutic proteins.

The glycosylation of PSGL-1/mIgG_{2b} may be tailored by producing the protein in genetically engineered cell lines. Rational glycan design is achieved by transfecting cells with plasmids encoding PSGL-1/mIgG_{2b} together with specific glycosyltransferases that expand the glycosylation capacity of the cells.

In paper II, genetically engineered Chinese Hamster Ovary (CHO) cells were used to produce PSGL-1/mIgG_{2b} carrying blood group A and B determinants on type 1, 2 and 3 outer core saccharide chains. The multivalent mucins could adsorb chain type-specific anti-A antibodies, which indicate a prospective use of the mucins in immunoadsorption (IA) columns. IA columns are used to remove anti-A and anti-B reactive antibodies prior to organ transplantation across the blood group ABO barrier.

In paper III and IV, genetically engineered CHO cells were used to produce high affinity binders of Shiga toxin 1 and 2 (Stx1 and Stx2) and avian influenza hemagglutinin (H5). Biacore biosensor assays indicated that PSGL-1/mIgG_{2b} carrying the blood group P1 determinant in multiple copies bound with high affinity to Stx1 and Stx2, while PSGL-1/mIgG_{2b} presenting multiple copies of Siaα2,3Gal on different *O*-linked cores bound with high affinity to avian influenza H5. It remains to be shown if PSGL-1/mIgG_{2b} can competitively inhibit and sterically block toxin and viral attachment to the cell surface.

In conclusion, PSGL-1/mIgG_{2b} carrying specific carbohydrates is a versatile tool that can be used in a range of applications where the multivalency confers a biologically relevant binding.

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