UMEÅ UNIVERSITET

Development and Evaluation of Tools to Explore Posttranslational HexNAc-Tyrosine and Mucin-Type *O*-Glycosylation

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i Glasburen, Kemiskt Biologiskt Centrum,
fredagen den 1 oktober, kl. 09:00.
Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Professor, PhD, Carmen Galan
University of Bristol, UK

Kemiska institutionen/Department of Chemisty
Umeå universitet/Umeå university
Umeå 2021

Organization
Umeå University
Department name

Document type
Doctoral thesis

Date of publication
10 September 2021

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Title
Development and Evaluation of Tools to Explore Posttranslational HexNAc-Tyrosine and Mucin-Type *O*-Glycosylation

Abstract
Glycosylation is the most abundant form of post-translational modification (PTM). Recently, *O*-glycosylation attracted much attention in the glycoproteomic field due to its association with various diseases, such as pathogenic infections and cancer. However, glycoproteomic analysis of *O*-linked glycosylation is highly challenging due its structural diversity and complexity. New and efficient methods need to be developed to obtain a better understanding of the biological functions of *O*-glycans. In the presented thesis, glycopeptide microarrays were used as tools to explore the role of mucin type *O*-glycosylation in cancer, bacterial adhesion processes and galectin recognition on a molecular level, and to get insights into a new group of tyrosine *O*-glycosylation. A better understanding of these carbohydrate-protein interactions on a molecular level could facilitate the development of glycomimetic inhibitors to fight bacterial infections or block glycan binding proteins involved in cancer progression, or improve the design of novel carbohydrate-based cancer vaccines.
In the first part of this work, tools were developed to elucidate the role of a novel group of PTMs, where *N*-acetylhexosamine (HexNAc = α-GalNAc, α- or β-GlcNAc) was found to modify the hydroxyl group of tyrosine. Synthetic glycopeptides carrying this new modification, as well as glycopeptide microarray libraries were prepared to evaluate the abilities of plant lectins (carbohydrate-binding proteins) to detect HexNAc-*O*-Tyr modifications. These lectins are commonly used in glycoproteomic work flows to detect and enrich glycopeptides and -proteins. Additionally, HexNAc-*O*-Tyr-specific rabbit antibodies were raised and immunologically analyzed by enzyme-linked immunosorbent assays, western blot and microarray binding studies.
In the second part of the presented thesis, synthetic mucin glycopeptide microarray libraries were prepared and employed to explore carbohydrate-protein interactions of galectins, bacterial lectins and tumor specific antibodies. Mucin glycoproteins are part of the mucus barrier that protects the host against invading pathogens. However, bacteria and viruses have co-evolved with the human host and have developed strategies to promote virulence, for example by adhering to glycans on the host cell-surface. To combat bacterial infections, their virulence and pathogenicity must be understood on a molecular level. In this work, mucin glycopeptides were enzymatically modified with different fucose motifs and used to determine the fine binding specificities of fucose-recognizing lectins LecB from *Pseudomonas aeruginosa* and the *Clostridium difficile* toxin A. Furthermore, a synthesis strategy was developed to generate simplified mucin core glycopeptides that could be used as scaffolds to enzymatically generate LacdiNAc modified glycopeptides. They could be used in microarray binding studies to evaluate the glycan binding preferences of various proteins, including the *Helicobacter pylori* lectin LabA and human galectins, which play roles in cancer development and progression. Aberrant glycosylation of mucin glycoproteins has been associated with various types of cancer. Tumor specific carbohydrate antigens on mucins represent attractive antigenic targets for the development of effective anti-cancer vaccines. In this work, antibodies induced by tumor-associated MUC1 glycopeptide-bacteriophage Qβ vaccine conjugates were immunologically analyzed using MUC1 glycopeptide microarray libraries.

Keywords
Glycopeptides, mucins, mucin type-*O*-glycosylation, microarrays, tyrosine-*O*-HexNAcylation, lectins, MUC1 cancer vaccines, TcdA, LecB, galectins, LacdiNAc, antibodies

Language
English

ISBN
978-91-7855-646-5
978-91-7855-647-2

Number of pages
171 + 7 papers