Glycosidases as an analytical tool in glycomics assays

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Addendum

Summary

A large repertoire of medical and clinical research has extensively demonstrated the importance of glycosylation in human health. This has created a demand for the development of precision glycomics assays. These assays are not only valuable in academic research, but have immense potential for high-throughput industrial applications of quality control, quality assurance and contract-research glycan profiling. Precision glycomics assays are often based on chromatographic and mass spectrometric techniques which require instrumentation infrastructure and technical skills that are not always readily available. This creates a demand in industry for the development of glycomics assays that have a low infrastructure cost and that are user-friendly with minimal training requirements.

Chapter 1 of this thesis provides an introduction of disease-associated changes in protein *N*-glycosylation observed in human plasma and the various analytical techniques used for their analysis. A description is given of benefits and challenges of chromatography and mass spectrometry techniques widely used in industrial glycan profiling. These applications often use glycoenzymes, and the roles of these enzymes as an analytical tool in glycomics assays are described. Chapter 1 is concluded by defining the scope of this thesis which, in a nutshell, is the development of novel high-throughput glycomics assays via a re-fashioned approach to exoglycosidase-based glycan profiling.

The assays developed in this thesis are highly reliant on the exoglycosidases for their accuracy and precision. Thus, great importance was given to the sourcing of exoglycosidases of certain desired traits. Chapter 2 outlines the workflow for the screening of exoglycosidases from natural sources. A metagenomic study of a gut bacteriome highlighted certain genes that coded for antennary fucosidases. These putative enzymes were recombinantly expressed and shown to indeed have an antennary fucosidase activity by a commonly used industrial liquid chromatography-mass spectrometry technique. Remarkably, one of the enzymes was capable of removing the antennary α(1–3/4) fucosyl linkage from sialylated arms of *N*-glycans which makes it an interesting tool in glycan profiling. This was a novel and previously unreported activity for an antennary fucosidase. An in-depth structural study of the enzyme revealed the mechanism for its novel activity.

Sourcing novel enzymes for desired traits and understanding their specificity is not always as straightforward as exemplified in chapter 2. Chapter 3 outlines such a scenario in the sourcing of an *N*-acetylglucosaminidase that has potential for industrial applications. This enzyme was reported to specifically remove the bisecting *N*-acetylglucosamine residues from *N*-glycans which makes it novel in its activity. Such an activity has importance in glycan profiling. A structural study of the *N*-acetylglucosaminidase was performed to understand its mechanism and specificity. However, this understanding remained inconclusive and hence further research into the mechanism is required.

The predominant goal of this thesis is the development of exoglycosidase-based glycomics assays which have potential for industrial applications and commercialization. These assays were developed with a stern backbone i.e. they should be high-throughput, user friendly, cost-effective and have commercialization potential as analytical kits. Furthermore, these assays should provide a unique approach to an important analytical challenge.

The first analytical challenge identified was the quantification of antennary fucosylation in human plasma *N*-glycosylation. This glycosylation trait is often associated with inflammatory diseases such as autoimmune diseases, diabetes and cancer, thus having relevance in medical sciences. The industrial techniques used for the quantification of antennary fucosylation are based on HILIC(LC)-FLD/MS^n. Often these chromatographic techniques take several minutes up to an hour for analysis of a single sample. In this respect, MALDI-TOF-MS is the superior techniques when it comes to high-throughput analysis. Chapter 4 introduces a novel MALDI-TOF-MS based assay for the quantification of antennary fucosylation on human plasma *N*-glycosylation. The core fucosylation of *N*-glycans was depleted by exoglycosidases and the remaining antennary fucosylation was quantified. By combination of this workflow with sialic acid derivatization, sialyl Lewis X/A epitopes could be quantified. This MALDI-based assay not only outperformed an industrial LC-FLD-MS^n technique, but also significantly reduced the required time for analysis which is an important desirable trait in busy industrial analytical laboratories.

The second analytical challenge was the development of spectrophotometric assays instead of the previous combinations of chromatography and mass spectrometry. This type of assays has commercial interest as they are easily producible as kits and can cater to a large clientele of laboratories that are not equipped for routine high-end glycomics analysis. Chapter 5 introduces a novel plate-based spectrophotometric assay for the quantification of galactosylation and sialylation on human antibodies. Exoglycosidases are used for releasing sialic acids and galactose residues from the glycoproteins. These released residues are subjected to a redox reaction that produces a fluorescence signal which is quantified. This assay was shown to perform equally well as an industrial LC-FLD-MS^n technique for glycan profiling of antibodies, whilst not requiring the infrastructure or skills of an MS-based glycomics laboratory.

Finally, the analytical challenges for quantifying *N*-glycosylation are discussed in chapter 6 with respect to assay development. Firstly, the widely used techniques of chromatography, electrophoresis and mass spectrometry are discussed along with the less common techniques of glycan/lectin microarrays. The general pros and cons for these techniques are outlined and evaluated. Next, the most important and critical characteristics of glycomics assays for industrial applications are discussed in detail. Many of these assays are based on LC-FLD/-MS^n techniques and their molecular identification options are discussed in-depth. This includes collision induced dissociation, derivatisation and exoglycosidase-based profiling. Finally, the importance of glycosidases in industry is discussed, especially the need for sourcing and/or engineering exoglycosidase as analytical tools in industrial glycan profiling applications.