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SYNTHESIS OF *N*-GLYCANS FOR IMMUNOLOGICAL STUDIES

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ABSTRACT

Over the past few decades, immunologists have begun to try and develop vaccines against tumour cells. These vaccines typically contain an antigen that is present only on the tumour, which can then activate tumour-specific cytotoxic T-cells. One of several approaches to developing an effective vaccine involves targeting an endocytic receptor on antigen-presenting cells, which results in enhanced antigen cross-presentation. In these studies, the mannose receptor was used as an endocytic receptor. The mannose receptor (MR) can function in two ways on antigen-presenting cells; either to enhance general antigen uptake and/or to promote cross-presentation of the antigen.To date, there is no clear picture of the factors that are important in controlling antigen cross-presentation. The main aim of this project was to determine which structural parameters of the glycoprotein-antigen conjugate resulted in enhanced cross-presentation upon MR-ligation. This thesis therefore concerns the chemoenzymatic synthesis of defined glycopeptides and glycoproteins as chemical biology tools to help unravel the role(s) of the MR in antigen cross presentation. Herein N-glycans were produced either via total or semi-synthesis, and then enzymatically or chemically coupled to give homogeneous glycopeptides and glycoproteins.Enzymatic degradation of locust bean gum provided a Manβ(1→4)Man disaccharide building block which in turn allowed the synthesis of N-glycan disaccharide and tetrasaccharide oxazolines. This synthetic route was considerably shorter than all other previously reported syntheses of these two compounds. In addition, large N-glycan oligosaccharide oxazolines, $Man_9GlcNAc$-high mannose (from soybean) and sialoglycan-complex (from egg yolk), were accessed by semi-synthetic approaches.GlcNAc-Asn and GlcNAz-Asn were employed as model acceptors to represent the minimal structure of N-linked glycoproteins; the disaccharide, tetrasaccharide, Man9GlcNAc, and sialoglycan oxazolines were all used as donors. The structurally modified GlcNAz acceptor (Fmoc-Asn-GlcNAz) was found to be a suitable acceptor substrate for glycosylation catalysed by endo-β-N-acetyl-glucosaminidase (ENGase) enzymes, indicating that in future its incorporation into larger peptides may provide access to bio-orthogonally tagged antigens and allow more detailed investigations of antigen cross-presentation biology.Native glycoforms of immunological probe peptides were made by the use of ENGase enzymes, which attached sugar oxazolines to a peptide (OVA247-264A5K) containing a GlcNAc handle. Non-native glycoforms of the same probes were made by the use of click chemistry, to attach sugars to peptides (OVA247-264A5K) which contained a propargyl handle. Finally, glycoprotein remodelling of ovalbumin (OVA) was achieved with the N-glycan tetrasaccharide oxazoline donor using WT Endo A as catalyst. The synthesis of these glycopeptides and glycoproteins in homogenous form should facilitate future analysis to help define the pathway taken by an antigen after uptake by the MR.

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