Project: Chemoenzymatic glycan remodelling

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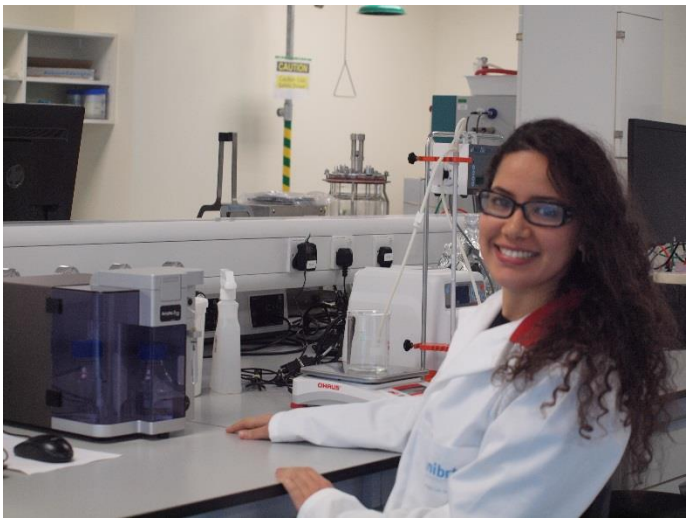

Background:
Several areas of a bioprocess are critical for the control of glycosylation
(a) Cell line: Introduce a gene to overexpress a specific transferase enzyme (Raymond et al 2015)
(b) Control of substrates in media (Villacres et al 2015)
(c) Remodelling preformed glycoprotein (Tayi and Butler, 2015; Tayi and Butler, 2017)

Proposal
(a) Optimize reaction conditions for targeted glycoform production by enzymatic modification
(b) Determine receptor binding capacity of specific glycoforms produced.
(c) Focus on defucosylation using a novel fucosidase for enzymatic remodelling or use a fucose transferase inhibitor in the bioprocess. Establish the relationship between fucosylated glycans and Fc receptor binding.

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Tayi,VS and Butler,M.. Solid-phase enzymatic remodeling produces high yields of single glycoform antibodies. Biotechnology Journal 2017 DOI: 10.1002/biot.201700381

Design of Single Glycoform Antibodies using Solid Phase Enzymatic Remodelling

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Introduction

The therapeutic efficacy of monoclonal antibodies (mAbs) to treat disorders such as cancer, autoimmune, and infectious diseases depends dramatically on their glycosylation profile, i.e. the structure of N-glycans attached to the Fc region. The modulation of their biological activity (e.g. ADCC and CDC), is critically influenced by these N-glycan compositions. Therefore, the production of mAbs with homogeneous glycan composition should be of primary interest to the biopharmaceutical industry, as it results in molecules with greatly higher clinical efficacy. In order to obtain homogeneity in the mAbs glycosylation profile, many approaches, such as sequential enzymatic modifications and solid phase transformation, have been developed. The newly reported method, by Tayi and Butler, has proven to efficiently produce single glycoforms antibodies which are thought to exhibit greater biological efficacy.[1] This method is advantageous when compared with standard methods as there is no need for intermediate purification of the antibody.
This project aims to optimise the method further using additional enzymes which are smaller and capable of catalysing a broader number of glycan modifications, ultimately resulting in a wider range of desirable single glycoforms. Once optimised the method will be verified, scaled up and established as a standard protocol.

Project Plan

Production of mAbs - Harvest mAb from bench scale bioreactor → Mab purification and remodelling the glycan profile enzymatically → Analysis and comparison of glycans by HPLC/CE/MS → Test the biological and binding properties of the resulting antibodies

Materials & Methods

Table 1: Enzymes used for enzymatic remodelling of glycans

Enzyme	Glycan Modification
Galactosyltransferase	Addition of galactose molecule
Sialyltransferase	Addition of sialic acid molecules
Galactosidase	Cleavage of galactose molecules
Sialidase	Cleavage of sialic acid residues
β-N-Acetylglucosaminidase	Cleavage of β-N-Acetylglucosamine residues
Fucosidase	Cleavage of fucose residues

Figure 1: Scheme of method for enzymatic modification of antibody to obtain single glycoforms[2]

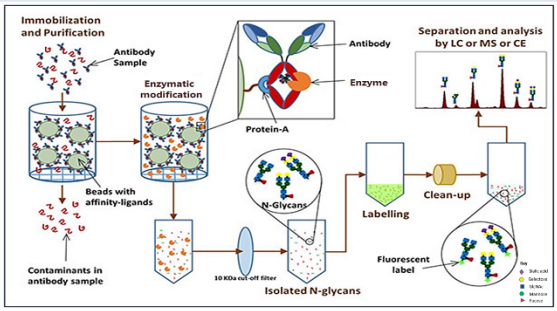

Table 2: Glycan characteristic and effect on antibody function

Glycan structure	Function	Example of structure
High mannose structure	Faster clearance from serum[3]	
Absence of fucose	Enhanced ADCC[4]	
Terminal galactose	Enhanced binding affinity to Fcγ RIIIa receptors[5]→ higher ADCC activity[6]	
Absence of terminal galactose	Reduced CDC activity[7]	
Terminal Sialic acid	Reduced ADCC activity.[8] Increased anti-inflammatory property[9]	

Conclusions

The new reported method[1] has proved to efficiently produce single glycoforms antibodies, which may present a higher efficacy. The challenge now is to optimise this method using new enzymes, smaller and capable of catalysing a broader number of glycan modifications, in order to obtain a higher yield of the required single glycoforms. After optimisation, the method could be scaled up and used as a standard purification protocol.

References

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