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Research paper

Glycosylation profiles of therapeutic antibody pharmaceuticals

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ABSTRACT

Recombinant antibodies specific for human targets are often used as therapeutics and represent a major class of drug products. Their therapeutic efficacy depends on the formation of antibody complexes resulting in the elimination of a target molecule or the modulation of specific signalling pathways. The physiological effects of antibody therapeutics are known to depend on the structural characteristics of the antibody molecule, specifically on the glycosylation which is the result of posttranslational modifications. Hence, production of therapeutic antibodies with a defined and consistent glycoform profile is needed which still remains a considerable challenge to the biopharmaceutical industry.

To provide an insight into the industries capability to control their manufacturing process and to provide antibodies of highest quality, we conducted a market surveillance study and compared major oligosaccharide profiles of a number of monoclonal antibody pharmaceuticals sampled on the Swiss market.

Product lot-to-lot variability was found to be generally low, suggesting that a majority of manufacturers have implemented high quality standards in their production processes. However, proportions of G0, G1 and G2 core-fucosylated chains derived from different products varied considerably and showed a bias towards the immature agalactosidated G0 form. Interestingly, differences in glycosylation caused by the production cell type seem to be of less importance compared with process related parameters such as cell growth.

1. Introduction

Immunoglobulins are serum proteins that play a central role in the humoral immune response by binding and inactivating antigens or triggering inflammatory or other cellular responses. The antibody core structure is composed of two identical light chains and heavy chains. They form a Y-shaped structure consisting of two identical antigen-binding domains (Fab) and an antibody crystallisable domain (Fc) with the heavy chain constant domains. All antibodies contain N-linked carbohydrate structures at conserved positions in the constant regions of the heavy chains. IgG, the most abundant in serum, has a single N-linked biantennary moiety at Asn297 following the consensus sequence N-X-S/T (X ≠ P), which is located in both heavy chains in the Fc region of the molecule [1]. In addition, IgG may possess Fab-associated carbohydrates with considerably varying structures [2].

Recombinant immunoglobulins are emerging as powerful pharmaceuticals for the therapy of a wide variety of diseases such as cancer or rheumatoid disorders. Several therapeutic and diagnostic monoclonal antibodies have been approved for market authorisation, and hundreds are in clinical trials or under development. Candidates in the production pipelines may be recruited by selecting or modifying epitope specificities or enhancing Fc effector functions [3]. All currently licensed antibody therapeutics are of the IgG class and have been manufactured using recombinant DNA technology in mammalian cell lines such as Chinese hamster ovary (CHO), mouse NS0 or SP2/0 myeloma cells. Fc glycosylation is essential to the structural integrity of the molecule [4,5], and alterations in glycosylation patterns due to differences in production conditions have been reported to affect susceptibility to proteolytic degradation, clearance rate *in vivo*, Fcγ receptor binding and activation, antibody-dependent cellular cytotoxicity (ADCC) and C1q component binding mediated complement activation [6–9]. Alterations in glycosylation could therefore compromise effector functions including bioactivity, clinical efficacy, pharmacokinetics, safety, stability and antigenicity [10,11]. Hence, glycosylation of recombinant IgG antibody molecules should optimally encompass naturally occurring oligosaccharides structures to avoid unexpected consequences. The challenge is to define expression systems that allow production of antibodies with defined structural and functional properties and Fc glycosylation patterns similar to native human IgG antibodies.

For the regulatory purpose [12,13], manufacturers are required to describe the degree of heterogeneity of their products in relation to the manufacturing process and should demonstrate comparability with clinical or preclinical batches, respectively. The structural characterisation of the molecule including the determination of the position and the nature of oligosaccharide subunits is of utmost

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importance for understanding the structure–function relationship and constitutes a precondition to establish product consistency. Whenever changes in glycosylation occur (e.g. after process changes) they may affect biological, immunochemical or physiochemical properties of the active ingredient and comparability should be confirmed.

We conducted a market surveillance study which provides an on-site comparison of major oligosaccharide glycosylation of therapeutic monoclonal antibody preparations currently licensed for the Swiss market. The glycosylation profiling data are evaluated regarding effects caused by the usage of different production cell lines and production conditions.

2. Materials and methods

2.1. Antibodies and mAb pharmaceutical preparations

Native IgG_1 fractions purified from human and mouse myeloma preparations were purchased from Calbiochem. Redimune® NF-Liquid, a polyvalent human normal immunoglobulin (IgG ⩾ 96%), was obtained from CSL Behring. Formulated drug products and purified cell culture supernatant bulk materials of 16 licensed antibody pharmaceuticals (A–P) were obtained from their respective manufacturers.

2.2. Enzymatic release and fluorescent derivatisation of N-linked oligosaccharides

Before cleavage, samples were desalted by centrifugation through a 30 kDa cut-off membrane (Microcon, Millipore), followed by one wash with peptide-N-glycosidase F (PNGase F) incubation buffer (20 mM sodium phosphate, 50 mM ethylenediaminetetraacetic acid (EDTA), 0.02 (w/v) sodium azide, pH 7.5). Oligosaccharides were released from the protein backbone with PNGase F for 15 h at 37 °C. Proteins were precipitated by heating to 95 °C for 5 min. After centrifugation, oligosaccharide solutions were dried in a SpeedVac. Twenty micro litres labelling reagent (500 µl 15% HAc was added to 5 mg 8-aminopyrene-1,3,6-trisulfonic acid (APTS) labelling dye) and 5 µl 1 M sodium cyanoborohydride/tetrahydrofuran (THF) were added to the pellets and incubated at 55 °C for 2 h. To stop the reaction, 500 µl water was added to the samples.

2.3. Capillary electrophoresis of APTS-labelled oligosaccharides

Cleaved and labelled oligosaccharides have the same charge and were separated based on their size and/or structure using a ProteomeLab PA 800 system (Beckman Coulter) equipped with an argon-laser fluorescence detector (Excitation: 488 nm, Emission: 520 nm). Separations were performed using a eCap N-CHO coated capillary (50 µm i.d., 40 cm effective length, 50 cm total length) in Carbohydrate Separation Buffer (Beckman Coulter). Sample solutions were introduced to the capillary by pressure (0.5 psi for 6 s). Analysis was performed for 15 min at 30 kV. Peak areas were corrected for labelling efficiency using an internal standard (IS), and relative amounts of G0, G1 (1–6), G1 (1–3) and G2 glycans were calculated.

3. Results and discussion

3.1. Characterisation of the mAb glycoforms

Human native IgG is composed of predominately N-linked Fc complex biantennary type oligosaccharides with heterogeneity in core fucosylation, terminal sialylation and galactosylation. This

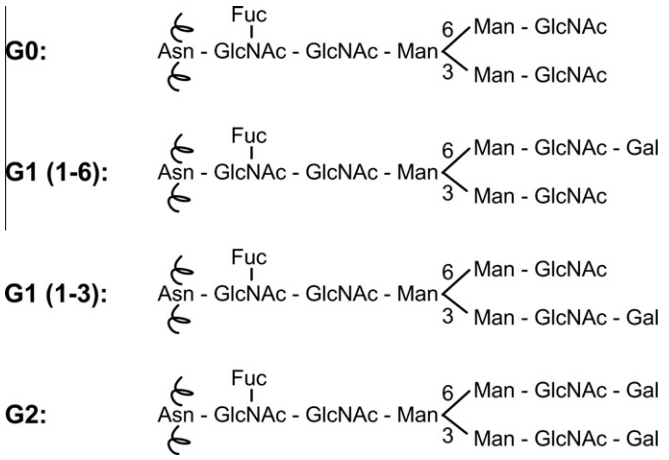

Fig. 1. List of the major N-linked oligosaccharides in IgG antibodies.

neutral (asialo) oligosaccharide structure is characterised by a fucose moiety attached to the innermost N-acetylglucosamine (GlcNAc) residue which in turn is covalently bound to the immunoglobulin asparagine (Asn) residue (Fig. 1). The G0 form lacks both galactose (Gal) residues at the ends of the biantennary chains. G1 (1–6) and G1 (1–3) are biantennary positional isomers carrying one Gal residue attached to the mannose GlcNAc branch. In G2, both branches carry a Gal residue. Recombinant IgGs show

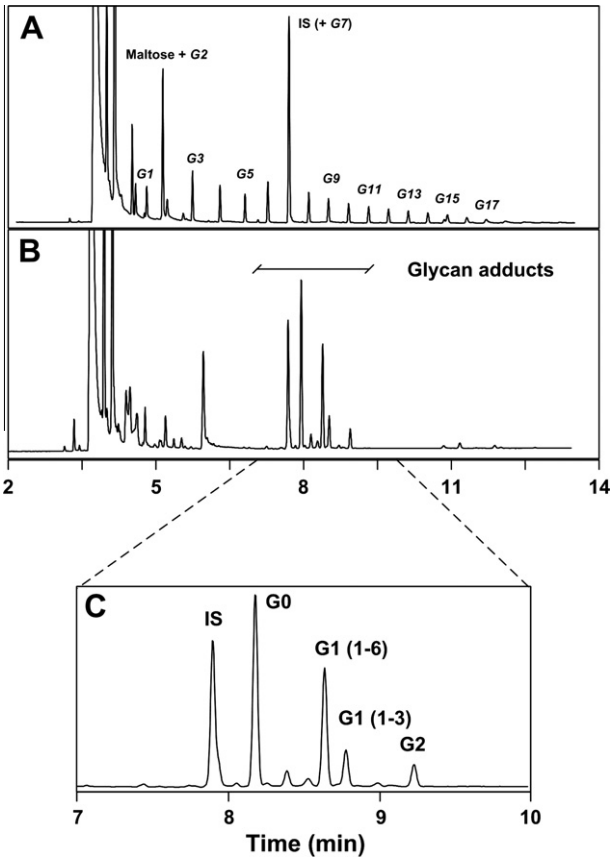

Fig. 2. Representative electropherograms of (A) APTS-labelled glucose ladder standard (*G1–G17*) spiked with maltose and maltoheptaose (IS). (B and C) PNGase F released N-linked glycan adducts of human IgG spiked with maltose and maltoheptaose (IS) and separated by capillary electrophoresis. Identification of relevant IgG oligosaccharides is described in Section 3.