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HPLC Strategies for Profiling and Sequencing Oligosaccharides

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11.1 Introduction

The sugars released from a pure glycoprotein often consist of a heterogeneous population containing both neutral and charged oligosaccharides. For example, the single N-glycosylation site in human erythrocyte CD59 is associated with more than 100 neutral and sialylated complex glycans, each representing a different glycoform (1). The existence of such extensive heterogeneity in biologically important glycoproteins requires refined approaches to the analysis of oligosaccharides. The adaptable technology which is described here represents a significant advance towards faster, more automated and more detailed strategies for the rapid profiling and analysis of sugars. Such technologies may be required for major studies, such as the human genome project, which defines DNA in normal and diseased states, and the proteome project, which sets out to analyse the total amount of protein in a living cell. It is worthy of note that genetic diseases are not caused by the genes themselves, but by the products for which the genes code and their post-translational modifications, which include glycosylation. In this chapter two strategies for rapid oligosaccharide analysis are described: Oligosaccharide profiling and detailed structural analysis.

Nomenclature for describing oligosaccharide structures: A(1,2,3,4) indicates the number of antennae linked to the trimannosyl core; G(0–4) indicates the number of terminal galactose residues in the structure; F: core fucose; F2: core fucose and one outer-arm fucose; F3: core fucose and two outer-arm fucoses, B: bisecting N-acetyl glucosamine (GlcNAc); S: sialic acid; Gal: galactose; M, Man: mannose, N: additional outer-arm N-acetyl hexosamine, G: additional outer-arm galactose. Outer arms are distinguished 1,3 and 1,6 according to the linkage of the mannose to the Man-1 core. Thus, A2G2FBS1(1,6) indicates a biantennary structure with core fucose, bisecting N-acetylglucosamine and one sialic acid attached to the galactose on the 1,6 arm.
Oligosaccharide profiling

This involves using a rapid, high-resolution, sensitive and reproducible separations technology which can give a detailed profile of the overall glycosylation of a protein. The methods described here are suitable for comparative fingerprinting of heterogeneous mixtures of both neutral and acidic glycans. The methods have many applications, which include monitoring the glycosylation of different batches of recombinant glycoproteins, analysis of glycosylation changes in disease and elucidation of the saccharide components of monoclonal antibodies.

Detailed structural analysis

The normal phase high-performance liquid chromatography (HPLC) conditions described here enable the profiles of total glycan pools to be interpreted directly to predict the structures of both neutral and acidic glycans from a single HPLC run. HPLC analyses of the products of simultaneous exoglycosidase digestions on aliquots of the total glycan pools are used to confirm the predicted structures without the need to isolate individual sugars. Additional information may be obtained by separating the glycan mixtures using a weak anion-exchange (WAX) HPLC column which separates the glycan populations according to charge. Finally, a reversed-phase HPLC system is described which provides a second-dimension chromatographic step which may separate oligosaccharides that co-elute on normal phase. Several general applications of the technology are described in section 11.4, illustrated with references to the following specific examples: (i) rapid profiling and fingerprinting of normal and rheumatoid arthritis (RA) IgG glycans, (ii) simultaneous sequencing of the total N-glycan pool released from normal human serum transferrin (HTf), (iii) characterization of the total glycan pool from HuE CD59, (iv) the two-dimensional separation of CD59 N-linked glycans according to charge (WAX) and subsequently analysed by normal phase HPLC, (v) resolution of sialylated oligosaccharide structures from fetuin, (vi) determination of the arm specificity of fucose residues in glycans from human parotid gland and (vii) the use of reversed-phase HPLC as a second dimension technique to resolve parotid glycans which elute as overlapping peaks on normal phase.