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Journal of Chromatography B, 825 (2005) 124–133
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Protein glycosylation analysis by liquid chromatography–mass spectrometry

Manfred Wuhrer*, André M. Deelder, Cornelis H. Hokke

Biomolecular Mass Spectrometry Unit, Department of Parasitology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands

Received 7 December 2004; accepted 28 January 2005
Available online 11 February 2005

Abstract

Liquid chromatography (LC)–mass spectrometry (MS) has developed into an invaluable technology for the analysis of protein glycosylation. This review focuses on the recent developments in LC and combinations thereof with MS for this field of research. Recently introduced methods for the structural analysis of released glycans (native or derivatised) as well as glycopeptides, on normal phase, reverse phase and graphitized carbon LC columns with online MS(/MS) will be reviewed. Performed on nano-scale or capillary-scale, these LC–MS methods operate at femtomole sensitivity and support the further integration of glycosylation analysis in proteomics methodology.

Keywords: Glycopeptide; Glycoprotein; Graphitized carbon; Normal phase; Reverse phase; Sensitivity

Contents

* Corresponding author. Tel.: +31 71 526 5077; fax: +31 71 526 6907.
E-mail address: m.wuhrer@lumc.nl (M. Wuhrer).

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doi:10.1016/j.jchromb.2005.01.030

1. Introduction

Protein glycosylation analysis has since long been a challenging task for the analytical biochemist. Compared to other classes of biomolecules, in particular peptides and lipids, the primary structure of glycans, either alone or in association with a protein, is not easily solved with a single technique. Variable composition, linkage, branching and anomericity of the constituent monosaccharides in combination with the general heterogeneity due to the indirect, non-template control of their biosynthesis are the basis of the structural complexity of glycoprotein glycans. As a consequence, a multitude of techniques is often necessary to fully determine the structure of a glycan. These techniques may include ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy (NMR) [1,2], gas chromatography (GC) [3] and liquid chromatography (LC) [4,5], electrophoresis [5] and mass spectrometry (MS) [6–8], all possibly in combination with chemical or enzymatic degradation and derivatisation methods.

Inherent to the complexity of protein glycosylation, the sensitivity with which it can be analysed, as well as the relatively extensive and time-consuming analytical procedures needed, have long been a limiting factor for applications in many biomedical and glycobiological studies. The maturating proteomics field requires better methods for the analysis of protein glycosylation. Partly responsible for the continuing progress in proteomics research are the technological developments in the MS field [9,10] and LC–MS based methods for glycosylation analysis have also benefited significantly from these developments in recent years.

Glycosylation of proteins can be analysed at different levels of detail, depending on the specific research question asked. In some cases it may be sufficient just to determine whether a protein is glycosylated or not. In order to get a more detailed picture of a glycoprotein however, glycosylation analysis may include the complete primary structure determination of all glycans on a given glycoprotein or glycoprotein mixture, including site-specific data on occupation and microheterogeneity of each N- and O-glycosylation site.

Glycosylation analysis is recognised as one of the main current challenges in proteomics [10], and in particular the integration of compatible approaches for proteomics and glycoproteomics in terms of scale, detail and sensitivity is a rapidly developing field. To achieve this compatibility, LC–MS based methods are invaluable. The combination of LC for the separation, and MS(/MS) for the detection and further structural analysis of glycans and glycopeptides provides detailed information at high sensitivity. Various mass analyzers (for example, ion trap (IT), quadrupole-time-of-flight, triple quadrupole) may be chosen for these analyses, each exhibiting specific advantages/disadvantages [11]. Although the mass spectrometer is an equally important factor in the combined LC–MS technology, this review will primarily focus on recent developments in miniaturized LC, which together with online nano-electrospray allow the sensitive LC–MS analysis of labelled or non-derivatised released glycans (see Section 2), and of glycopeptides (see Section 3), as schematically represented in Fig. 1.

2. Analysis of released glycans

The most convenient approach to determine the structures of the glycoprotein glycans present in a given protein preparation regardless of their position on the protein backbone(s) requires their release by enzymes (peptide N-glycosidases F and A, endo-glycosidases) or by chemical procedures (β-elimination or hydrazinolysis) [8]. The released glycans can then be analysed by LC–MS in their reducing form, as alditols, after permethylation, or after addition of a label to the reducing end. Typically, electrospray MS as well as MS/MS, on-line coupled to LC are widely applied to the analysis of oligosaccharide derivatives [7,8,12–14],

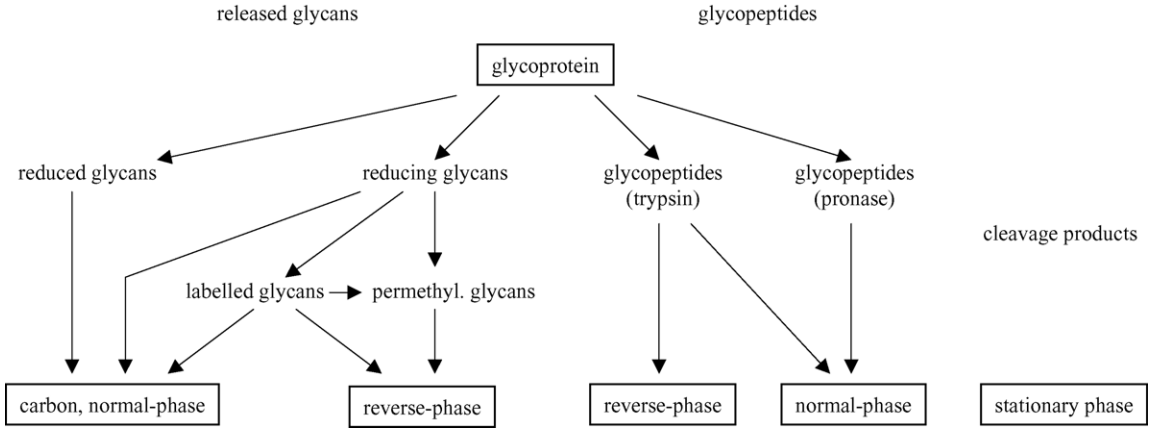

Fig. 1. LC–MS approaches for the analysis of protein glycosylation. Glycan release and protein degradation lead to various cleavage products, which can be analysed by LC–MS using different stationary phases. Enrichment techniques and approaches using deglycosylated peptides are not included in the scheme, permethyl, permethylated.