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Advances in Mass Spectrometry for Glycoscreening and Sequencing in Biomedical Research

Adrian Robu[1,2,4], Loredana Lupu[1,2,4], Roshanak Aslebagh[3], Alina D Zamfir[1,2]* and Costel C Darie[3]

[1]*Mass Spectrometry Laboratory, National Institute of Research-Development for Electrochemistry and Condensed Matter, Timisoara, Romania, Plautius Andronescu Str. 1, 300224, Timisoara, Romania*
[2]*Faculty of Physics, West University of Timisoara, Romania, Blvd. V. Parvan 4, 300223, Timisoara, Romania*
[3]*Biochemistry and Proteomics Group, Department of Chemistry and Biomolecular Science, Clarkson University, 8 Clarkson Avenue Potsdam, NY, 13699-5810, USA*
[4]*These authors contributed equally to this work*

Abstract

During the past several years, applications of Mass Spectrometry (MS) in the biomedical research increased considerably. While MS is for many years heavily used in proteomics, for protein identification and quantification as well as for biomarker discovery, for a long time its applications in glycomics were limited, mainly because of the challenging conditions required for the ionization and detection of most carbohydrate classes. However, due to the development of high performance analytical instrumentation, MS in particular with Electrospray (ESI) and Matrix Assisted Laser Desorption/Ionization (MALDI) started to be intensively applied also to the analysis of post-translational modifications such as glycosylation, acetylation or phosphorylation. Focus on MS-based glycosylation is, however, scarce. Therefore, analysis of glycoproteins in particular diseases through glycoscreening and sequencing is another new MS-based avenue, yet to be pursued. In this context, we discuss briefly here the recent advances of MS in glycomics and glycoscreening and their applications in biomedical research, with a particular emphasis on cancer, lysosomal storage and bacterial diseases.

Keywords: Glycosylation; Mass spectrometry; Oligosaccharides; Glycoscreening; Glycoconjugates

Introduction

Mass Spectrometry (MS) is an analytical method that can help in solving biomedical, biological, or biochemical problems. MS may also be used in protein or glycoprotein characterization, larger scale protein or metabolite characterization (i.e. proteomics, metabolomics), with applications in basic or applied research, in domains such as academia, biotechnology and pharmaceutical industry, forensic sciences, health, food sciences or pollution.

Glycomics is a rather new MS-based science that focuses on the identification and characterization of glycans as either free oligo-and polysaccharides or those who are attached to proteins (glycoproteomics) or lipids (glycolipidomics).

Recently, more and more biomedical discoveries in glycomics (both glycoproteomics and glycolipidomics) have been made using high performance MS and some research seem promising for discovering new biomarkers for different types of tumors or degenerative diseases [1-9].

In principle, a mass spectrometer contains three main components: the ion source, the mass analyzer and the ion detector. Due to recent developments and advances in MS technology [10-12], a powerful array of instruments has been created, mostly using a combination of more than one type of analyzers. Among the most popular instruments are the hybrid quadrupole time-of-flight MS (QTOF), triple quadrupole (QQQ), TOF-TOF MS, ion trap, Q-Trap, etc. All these combinations allow the analysis of a wide variety of molecules.

Lately, nanotechnology achievements contributed significantly to the improvement of ionization by development and introduction of chips for Electrospray Ionization (ESI). At the same time, the sequence coverage for proteins or glycans could be increased by using efficient MS fragmentation techniques such as Collision-Induced Dissociation (CID), Electron Transfer Dissociation (ETD), Electron Detachment Dissociation (EDD), Infrared Multiphoton Dissociation (IRMPD) etc. and a separation/fractionation procedure prior to ionization.

In glycoproteomics, modern High Performance Liquid Chromatography (HPLC) at lower flow rate such as Ultra-High Performance Liquid Chromatography (UPLC) can be used in direct coupling to ESI and nanoESI MS. However, in glycomics, when the sample is available in even lower amounts, alternative strategies for prefractionation of molecules such as microfluidic-based devices or capillary electrophoresis are needed.

A special role in the development of instrumentation with great processing power is played by the micro fabricated devices and modern technologies that have the ability to detect distribute and manipulate small amounts of samples.

A large number of studies on the development of bioanalytical applications that focus on analysis of glycans were published [13-19] . Below we discuss several of such examples with biomedical relevance.

Glycoanalysis for Biomedical Research

Glycoproteins are proteins which contain at least one glycan structure. Because of the multiple glycosylation sites on the proteins, of the various type of glycosylation (O- or N-glycosylation) and microheterogeneity, these biopolymers are very complex. Glycosylation [20] is one of the most important protein Post-Translational Modifications (PTMs) because it triggers many biological processes (i.e. cell-cell recognition) and influences protein biological activity.

In addition to glycosylation, other protein PTMs exist such as formation of disulfide bridges, acetylation or phosphorylation [21,22].

***Corresponding author:** Alina D Zamfir, Faculty of Physics, West University of Timisoara, Plautius Andronescu Street 1, 300224, Timisoara, Romania, Tel: 40-256-494-413; E-mail: alina.zamfir@uav.ro

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The last PTM listed (phosphorylation) is a reversible and common one that plays an important role in controlling and modifying a large number of cellular processes.

Nowadays, for reliable insight into the world of glycomics, an increased number of techniques are being developed for the study of complex glycans and glycoconjugates. Some glycans and glycoconjugates play an important role in the development of the human brain and therefore can be used as biomarkers for different pathological states at this level. Among them, sialylated glycosphingolipids (known as gangliosides) are complex structures found in diverse proportions in the human body but mostly concentrated in the central nervous system. Their expression, type and structure were found modified in pathological conditions, very often with modification patterns that are specific to the disease. Hence, the detailed MS-based mapping of ganglioside composition in specific regions of the brain in health and disease was shown to be a valuable methodology for early and reliable diagnosis of brain afflictions among which primary and secondary brain tumors and neurodegeneration [23,24].

In the past several years, the applications of MS to the field of glycomics for biomedical research have been tremendously extended. Glycans extracted from almost all human matrices including body fluids such as serum, cerebrospinal fluid, saliva, urine, milk etc. were subjected to MS analysis for screening, sequencing and biomarker discovery. For example, Nwosu et al. were among the first to use MS and tandem MS for a differential analysis of the species of glycans both from human and bovine milk. The project aimed at discovering the differences between both milk samples. The authors found hundreds of glycoform isomers and concluded that the primary difference between the human and bovine milk was the NeuGc residue, observed only in the bovine milk. The results indicated a high degree of sialylation and fucosylation in the human milk and a low fucosylation in the bovine milk. Overall, in this study, using a combination of nano-flow LC coupled with a QTOF mass spectrometer, the authors identified 38 species of N-glycans in the human milk and 51 in the bovine milk.

Numerous methods for the analysis of the specific glycosylation were designed to facilitate the development of a worldwide glycan database. Khatri et al. [25] presented a new system based on on-line Hydrophilic Interaction Chromatography (HILIC) enrichment trap for ionization of low abundant glycopeptides. This new LC-MS system, that allows the analysis of the glycopeptides and glycoproteins in a single step, was applied for human transferrin, human alpha-1-acid glycoproteins and influenza A virus to demonstrate its efficiency.

Among the O-glycans which exhibit a high biomedical relevance, glycosaminoglycans (GAGs) represent a particular category expressed in the extracellular matrix. The primary structure of several glycosaminoglycans is organized in repeating disaccharide units by segregation into alternating blocks of specific sulfation patterns that form oligosaccharide sequences with specific binding functions. Thus, GAGs form the extracellular environment, also called glycocalyx, in which they interact with different proteins such as growth factors and hormones. Due to their role in transmitting information from the extracellular side to the intracellular one, GAGs can be analyzed to detect pathological changes in various diseases such as cancer and cancer metastasis. For a better understanding of the GAGs expression and their role in pathogenesis, the researchers [26] developed a new method for their profiling at the histological scale. The authors have developed an approach based on Size Exclusion Chromatography (SEC) coupled to MS which was initially tested on digested Heparan Sulfate (HS) disaccharides extracted from bovine brain cells. The method could be successfully applied also on HS extracted from human glioblastoma and astrocytoma.

In another interesting study, Hu et al. [27] designed a novel algorithm called Heparan Sulfate Sequencing (HS-SEQ), for HS sequencing to be used in tandem MS (MS/MS). The authors tested the algorithm in Negative Electron Transfer Dissociation (NETD) on a pure synthetic saccharide and demonstrated that this new method provides highly accurate information in the interpretation of HS tandem mass spectra.

Recently, glycoscreening by MS emerged as a viable method also for rapid diagnosis of Lysosomal Storage Diseases (LSDs), with a particular focus on the identification and characterization of the glycans and glycoconjugates i.e. glycoproteins, which are biomarkers of the respective condition.

LSDs represent recessive disorders characterized by a genetic defect in one or more specific lysosomal enzymes, activator protein, or membrane protein, resulting in deficient enzymatic activity. Lysosomes contain hydrolases that reduce certain complex glycans into smaller components. If a specific lysosomal enzyme is not present in sufficient quantities or it is inactive, the substrate accumulates progressively, interrupts the cell functioning and triggers the disease. As LSDs are rare diseases, their approach by MS is a difficult task because of the limited number of patients and reduced availability of analytical material such as blood, cerebrospinal fluid and urine [28]. Nonetheless, currently a number of laboratories are focused on the development of MS-based methodologies for rapid and reliable diagnosis of LSDs.

Among LSDs, Schindler disease [29-31] is described as an autosomal recessive disorder caused by the deficient activity of α-N-acetylgalactosaminidase (α-NAGA), previously known as α-galactosidase B – a lysosomal hydrolase. In the case of this extremely severe condition, monitoring by advanced MS methods the glycopeptides excreted as catabolic products in patient urine became lately an option for rapid diagnosis. By employing an arsenal of high performance mass spectrometric [32,33] such as fully automated chip-nanoESI in combination with either QTOF or Fourier Transform Ion Cyclotron Resonance (FTICR) MS, capillary electrophoresis [34] in off- and on-line combination with MS it was revealed for the first time the modified expression and also the elevated concentrations of O-glycopeptides in patient urine as compared to healthy controls, from which a disease diagnosis could be established.

Mass spectrometric approaches were successfully introduced also for diagnosis and monitoring of Fabry disease, an inherited condition, caused by the absence or the diminished activity of the α-galactosidase A, which leads to the accumulation of Globotriaosilceramids (Gb3). The symptoms of this disease [35] appear as severe deterioration of cardiac and renal functions, strokes etc. Fabry disease has its onset in childhood, being characterized by acroparesthesia, angiokeratoma, corneal and lenticular opacity and hyperhidrosis. Several researchers [36] concluded that the substantial improvements in health-related quality of life can appear using long-term treatment with agalsidase beta.

In recent years, several MS-based strategies for monitoring the α-galactosidase A enzyme in Dried Blood Spots (DBS) [37,38], as well as for measurement of the storage products in plasma or urine have been developed. In 2013, a novel platform for rapid and reliable diagnostic of Fabry disease, based on enzyme assay and fully automated chip-nanoESI MS, CID and ETD MS/MS was reported [38].

The enzymatic assay workflow comprised five stages: i) obtaining the DBSs by uniform spotting of blood onto collection paper; ii) enzymatic

extraction from DBS using an aqueous buffer solution; iii) enzymatic reaction between the DBS α-galactosidase extracts and the substrate/internal standard; iv) purification steps of the final enzymatic mixture; v) fully automated chip-nanoESI MS and tandem MS analysis of the reaction products on a High Capacity Ion Trap (HCT) ultra PTM mass spectrometer coupled via an in-laboratory mounting system to a Nano Mate robot incorporating ESI 400 chip technology. The method enabled discrimination between different Fabry cases, an aspect with major clinical importance when choosing an appropriate therapy. This MS-based tool for rapid detection of rare diseases exhibited the capability to distinguish the situations in which the enzyme is completely absent from those in which the enzyme is present at the trace level, as well as between the patients unable to produce the enzyme from those that produce an inactive enzyme.

In human cancer, glycomics and glycoproteomics assays have generated many datasets of potential diagnostic, prognostic and therapeutic significance [39]. Initially, the glycoproteomics studies related to cancer have been based on advanced MS and 2D polyacrylamide gel electrophoresis (2D-PAGE). However, recent alternative technologies such as MS in on-line combination with reversed-phase HPLC, protein and glycan arrays/antibody microarrays or isotope-coded affinity tag technology and microfluidics as well as the development of the efficient fragmentation methods for tandem MS were optimized and successfully introduced in cancer research for determination of abnormal glycans/glycoproteins that have biomarker value [39,40].

Besides all these interesting applications, one of the most important goals of MS in glycomics and glycoproteomics is related to bacterial diseases. In this context, noteworthy to mention is the work of Lane et al. [41] who have been engaged in development of rapid methods for the screening of several complex oligosaccharide mixtures with potential anti-adhesive activity against bacteria. The methods were based on using the whole bacterial cells to "deplete" free oligosaccharides from solution. The depletion assay confirmed selective bacterial interaction with certain oligosaccharides.

Perspectives

Increasing the number of methods which can be effectively used for the identification and characterization of glycans, glycoproteins and glycolipids will hopefully aid in to early diagnosis of various pathologies and in monitoring their development or remission during therapy. The achievements described here demonstrate not only a progress in the field but also that glycoanalysis has clear perspectives for development into a routine procedure in clinical and biomedical practice.

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