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Methods in Comprehensive Mass Spectrometry-Based Measurement of Sphingolipids

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Abstract

Molecular analysis of lipids has essentially improved by introduction of multidimensional mass spectrometry. This important technique allows identification and quantification of complex sphingolipids, e.g. glycosphingolipids and ceramides, from diverse intestinal sources including liver and bowel. In the following a short overview is given concerning this important technical tool which facilitates molecular characterization of lipids in intestinal physiology and diseases.

Keywords: Ceramides; Glycosphingolipids; Lipids; Mass Spectrometry

Introduction

The term lipids describes a broad group of molecules with hydrophobic or amphiphilic nature including fatty acyls, glycerolipids, glycerophospholipids, sterol lipids, and sphingolipids which are widely distributed and associated with a plethora of biological functions including structural components, energy storage, and signaling cascades [1].

Important functions in cellular signaling are attributed to the family of sphingolipids with the major subclasses ceramides and sphingosines. These molecules are of high importance in regulation of cellular permeability, apoptosis, and cellular transformation. Although sphingolipids do not contain a glycerol group, they have a structural similarity to glycerophospholipids. The common structural feature, a sphingoid base backbone with eighteen carbon amino-alcohol structure, is converted into ceramides, phosphosphingolipids, and other species. Sphingolipids may be conveniently described in terms describing backbones, head groups, and sugar units [2]. Following biochemical pathways, the sphingoid base backbone is available by de novo synthesis from serine and a long-chain fatty acyl-CoA or by cleavage of sphingomyelin [3]. It is beyond the scope of this article to describe sphingolipid metabolism and biochemistry in detail, but the reader is referred to more specific articles on this subject [4].

The term complex sphingolipids describes the structural feature of sphingolipid species differing by both the order and type of sugar residues attached to their groups. In liver and intestine, sphingolipids are abundantly found. They are essential for structural integrity and act as bioactive messengers modifying diverse cellular activities including proliferation, differentiation, apoptosis, and inflammation [5]. In the gut, barrier function, cholesterol absorption, inflammation, and tumourigenesis are modified by sphingolipids [6].

Extraction of lipids from liver and intestinal tissues follows standard algorithms [7]. In general, tissues of interest should be macroscopically dissected (e.g. preparation of the mucosal layer) and afterwards homogenized without cryoconservation. An internal lipid marker must be added to the solution prior to the extraction of lipids with methanol and chloroform. Cryoconservation and storing of the extract until the measurements is possible.

This article will not in detail consider the many approaches and techniques that can determine and characterize lipids. The following sections give an overview of mass spectrometry-based lipid analysis and profiling of sphingolipids that are commonly used by lipidologists to investigate lipids in health and disease.

Principles of Mass Spectrometry-Based Lipid Analysis

To understand and analyze the impact of lipids in health and disease, mass spectrometry-based lipid analysis, so called 'lipidomics', has emerged as a promising tool in the last few decades. Lipidomics, the large-scale analysis of cellular lipid pathways and networks allows the study of the whole set of lipids of organisms/cells/tissues. It provides the scientists with methods to analytically distinguish different lipid species that are often metabolically interconvertible and structurally similar [8]. Because of the complexity of the lipidome, comprehensive techniques are required for elucidation of physiological properties and functionality of different lipid metabolites. Since mass spectrometry based techniques have become powerful tools in lipidomic methodology, today scientists have the opportunity to detect and quantify multiple lipids in a single sample [1,9].

The technical components of mass spectrometers always include an ion source to generate charged ions, a mass analyser to separate those ions by their mass to charge ratio, and a collector/detector to detect and quantify the analyzed sample. Two approaches that provide sensitivity and structural specificity in ion generation are Electro spray Ionization (ESI) and Matrix-Assisted Laser Desorption Ionization (MALDI) mass spectrometry (Figure 1). ESI is accomplished by continuously infusing a solution of lipids through a small capillary into an electric field, thereby generating very fine charged lipid droplets. These lipid droplets rapidly evaporate and divide into individual charged ions, as they travel along the electric field and enter the mass spectrometer, typically a triple quadrupole mass analyzer. This ionization technique results in a very soft ionization and yields primarily intact molecular species with little or no fragmentation. In contrast to ESI, MALDI mass spectrometry, typically applied in conjunction with time-of flight mass analyzers, is a laser-based soft ionization method that is most often used for protein analysis and only to a small extent for lipid analyses. In MALDI, the lipid sample is mixed with an organic matrix component and irritated with a laser to ionize the lipid molecules. After ionization, the analytes

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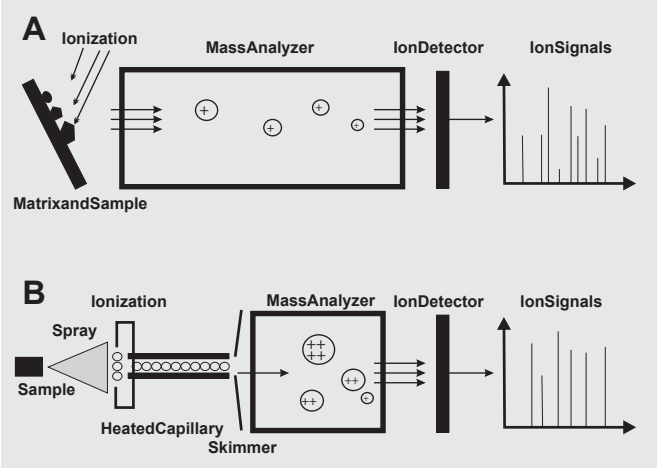

Figure 1: Principles of mass spectrometry. A: Matrix-assisted laser desorption ionization (MALDI) mass spectrometry is illustrated. Matrix loaded samples are ionized and further characterized with mass analyzer and ion detector; **B:** Electrospray ionization (ESI) mass spectrometry is accomplished by continuously infusing a sample solution into an electric field, generating very fine charged droplets with soft ionization and yields primarily intact molecular species with little or no fragmentation.

are accelerated out of the MALDI source into a mass analyzer. Matrix choice is critical for generation of intact molecular ions. One role of the matrix is to prevent analyte-analyte molecular interactions during the ionization process. Because of the nature of MALDI ionization, this technique has been successfully utilized for more complex lipids [10-13].

Furthermore, the power of MALDI technology lies in its application for tissue imaging mass spectrometry. This direct tissue analysis of lipid species provides the opportunity to identify not only quantities but also the distribution and subcellular localization of lipids within the tissue [14,15]. With this point of view MALDI overcomes other approaches, because almost all techniques used to identify and quantify lipids involve their extraction and removal from their biological source resulting in a loss of topological information.

Mass spectrometry provides many advantages in lipid analysis, especially sensitivity, specificity and structural information. The combination of state of the art tandem mass spectrometry techniques (MS/MS) and classical separation techniques such as high pressure liquid chromatography (HPLC) provides an additional criterion for specific identification of lipid species due to their retention time and also allows distinction of isomeric species. Thus, liquid chromatography-mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS)-based methods are currently one of the most popular technologies in lipid research [1,8,9].

Another mass spectrometric technique that is referred to as shotgun lipidomics works without direct coupling of any chromatography for lipid separation. Different from the LC-based lipid analysis, this direct infusion-based lipidomic technique allows absolute quantification of hundreds of lipid species in small quantities with high throughput. However, one significant limitation is the inability to distinguish isomeric and isobaric lipid species.

Collectively, numerous new lipidomic techniques have already been developed and more will come in the next years since their application for the identification of lipid pathways and functions, the investigation of lipid-mediated disorders and drug evaluation among others has been increasingly recognized [16-19].

Profiling Sphingolipids with Mass Spectrometry

Sphingolipidomics, a section of lipidomics that focuses on the large-scale analysis of the cellular sphingolipidome, is rapidly expanding. Due to the highly diverse and complex class of molecules, the emergence of comprehensive, structure specific and quantitative analyses of all sphingolipids is necessary to accurately distinguish the many different subclasses of sphingolipids. Elucidation of the roles of sphingolipids in cell structure, function and signaling is critical since this class of lipids comprises one of the largest numbers of bioactive lipid subspecies. The actual size of the sphingolipidome is not known so far, but it is considered to be immense.

Three basic approaches for an in depth and large-scale analysis of sphingolipids have been rapidly developing, the LC-MS- or LC-/MS/MS methods, the direct infusion-based shotgun-lipidomics and the MALDI imaging mass spectrometry. LC-MS/MS has been used to identify, quantify, and determine the structures of free sphingoid bases, free sphingoid base phosphates, ceramides, monohexosylceramides (both galactosylceramides and glucosylceramides), lactosylceramides, sphingomyelins and more complex glycosphingolipids [10,20-23]. Quehenberger and colleagues, for example, successfully employed LC-MS/MS to profile sphingolipids in human plasma. By combination of normal-phase HPLC and ESI-MS analysis, over 200 individual plasma sphingolipids were identified and quantified [24]. Direct infusion based shotgun lipidomics enables determination of the levels of many low-abundance sphingolipid metabolites e.g. ceramide-phosphates, sphinganine and sphingosinephosphates in a variety of biological samples [25,26]. In a recent study the determination of sphingosine levels in the lysosome was achieved using this method [25]. Although shotgun lipidomics has some limitations, these methods are very useful and efficient to quantify the molecular species of one entire lipid class from any unknown sample. Different from LC-MS/MS- and infusion-based techniques, MALDI tissue imaging has been applied to directly analyze tissues and cells for their lipid content and localization without loss of topological and sub-cellular information. Direct probing of brain slices *via* MALDI e.g. revealed that the cerebellar cortex contained low levels of sphingomyelins and sulfatides, but high levels of gangliosides whereas the cerebellar peduncle contained large amounts of sulfatides and smaller quantities of gangliosides [27]. In another tissue imaging study, Liu and colleagues observed that sulfatides were mainly located in ovarian carcinoma epithelium, but not in histologically normal ovarian epithelium [28]. The development of MALDI tissue imaging greatly enhances the application spectrum of sphingolipidomic analysis.

With the ongoing development of these powerful sphingolipidomic approaches the role of sphingolipids in cellular functions and pathologic states will become clearer, and the identification and establishment of preventive and therapeutic approaches will become more focused. Thus, sphingolipidomics should enable scientists to improve the understanding of the cellular sphingolipidome.

Concluding Remarks

Lipids are molecules of high structural diversity and function. Complex analysis of lipid tissue composition (lipidomics) is a powerful approach to get an *in vivo* structure-function relationship. In the field of lipid analysis, the innovating tool mass spectrometry is well established. In particular, mass spectrometry is of high importance for sphingolipid analysis from several biological sources.

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