Journal of Chromatography B, 877 (2009) 2600–2606

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Review

Recent progress in the analysis of uremic toxins by mass spectrometry☆

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ARTICLE INFO

Article history:
Received 6 September 2008
Accepted 20 November 2008
Available online 27 November 2008

Keywords:
Mass spectrometry
Uremic toxin
Modified proteins
Chronic kidney disease
Uremia

ABSTRACT

Mass spectrometry (MS) has been successfully applied for the identification and quantification of uremic toxins and uremia-associated modified proteins. This review focuses on recent progress in the analysis of uremic toxins by using MS. Uremic toxins include low-molecular-weight compounds (e.g., indoxyl sulfate, *p*-cresol sulfate, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, asymmetric dimethylarginine), middle-molecular-weight peptides, and proteins modified with advanced glycation and oxidation. These uremic toxins are considered to be involved in a variety of symptoms which may appear in patients with stage 5 chronic kidney disease. Based on MS analysis of these uremic toxins, the pathogenesis of the uremic symptoms will be elucidated to prevent and manage the symptoms.

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1. Introduction

This article reviews recent papers, particularly published after my previous review [1], on the use of mass spectrometry (MS) for the analysis of uremic toxins. The uremic syndrome is considered to be caused by an accumulation of uremic toxins due to kidney dysfunction. Ninety compounds have been considered to be uremic toxins [2]. Sixty-eight have a molecular-weight less than 500 Da, 12 exceed 12,000 Da, and 10 have a molecular-weight between 500 and 12,000 Da. Twenty-five solutes are protein-bound.

Recently, the research on uremic toxins arouses interest, even if the problem is an old matter, owing to the application of new analytical methodologies. Gas chromatography/mass spectrometry (GC/MS) has been successfully used for the analysis of low-molecular-weight compounds accumulated in uremic blood. The ionization methods, such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), have enabled the MS analysis of high-molecular-weight substances such as peptides and proteins. Liquid chromatography/electrospray ionization–mass spectrometry (LC/ESI–MS) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry

☆ This paper was presented at the 33rd Meeting of the Japanese Society for Biomedical Mass Spectrometry, Tokyo, Japan, 25–26 September 2008.

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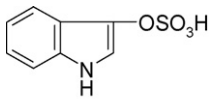
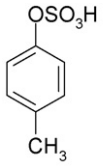
doi:10.1016/j.jchromb.2008.11.032



Indoxyl sulfate
(MW 213)

Fig. 1. Chemical structure of indoxyl sulfate.



p-Cresol sulfate
(MW 188)

Fig. 2. Chemical structure of *p*-cresol sulfate.

(MALDI-TOF-MS) are useful to characterize the structure of proteins modified with advanced glycation end products (AGEs) and oxidation. The modification of proteins is enhanced in uremic patients, and is considered to be responsible for some uremic symptoms.

Tandem MS (MS/MS) involves multiple steps of mass spectrometric selection, with fragmentation occurring in between the stages, and permits identification of peptide sequence by generating product ion spectra of selected precursor ion of a protein. MS/MS includes triple quadrupole (QQQ), time-of-flight/time-of-flight (TOF/TOF), quadrupole/time-of-flight (Q/TOF), and quadrupole/ion trap (Q/IT) analyzers. GC, high performance liquid chromatography (HPLC) or capillary electrophoresis (CE) can be directly combined with MS or MS/MS.

Ion mobility spectrometry–MS (IMS–MS) is a method that combines the features of ion mobility spectrometry and MS to identify different substances in a sample. An ion mobility spectrometer is a spectrometer capable of detecting and identifying very low concentrations of chemicals based upon the differential migration of gas phase ions through a homogeneous electric field. Ion mobility is a parameter that is dependent of ion mass, size, and shape. Thus, IMS, when coupled with MS, offers value-added data not possible from mass spectra alone [3].

Ambient MS has been developed using desorption electrospray ionization (DESI) [4] that enables to record mass spectra on ordinary samples, in their native environment, without sample preparation or preseparation by creating ions outside the instrument. In DESI, electrically charged droplets are directed at the ambient object of interest; they release ions from the surface, which are then vacuumed through the air into a conventional mass spectrometer.

Imaging MS has been developed as a technique for the direct tissue analysis of biomolecules including proteins using MALDI-MS, while preserving the abundance and spatial distribution of each analyte. It can be used to profile discrete cellular regions and obtain region-specific images, providing information on the relative abundance and spatial distribution of proteins, peptides, lipids, and drugs. Imaging MS technique has been applied to kidney disease and toxicity [5].

2. Low-molecular-weight uremic substances

2.1. Indoxyl sulfate

Niwa et al. [6,7] measured serum levels of indoxyl sulfate (Fig. 1) by internal-surface reversed-phase HPLC, and confirmed its identification by using MS. Indoxyl sulfate is a uremic toxin that accelerates the progression of chronic kidney disease, and is derived from dietary protein. A part of protein-derived tryptophan is metabolized into indole by tryptophanase of intestinal bacteria such as *Escherichia coli*. Indole is absorbed into the blood from the intestine, and is metabolized in the liver to indoxyl sulfate, which is normally excreted into urine. In chronic kidney disease, however, a decrease in renal clearance of indoxyl sulfate leads to its increased serum levels. The serum level of indoxyl sulfate is markedly increased in hemodialysis patients. Indoxyl sulfate cannot be removed effectively by conventional hemodialysis because of its binding to serum albumin. Indoxyl sulfate shows various uremic toxicity such as inhibition of drug binding to albumin, nephrotoxicity (progression of chronic kidney disease) [8,9], vascular toxicity (aortic calcification, vascular smooth muscle cell proliferation, and endothelial dysfunction) [10–12], and osteoblastic dysfunction accompanied by skeletal resistance to parathyroid hormone. Administration of an oral sorbent (AST-120) decreases the serum and urine levels of indoxyl sulfate, and inhibits the progression of chronic kidney disease.

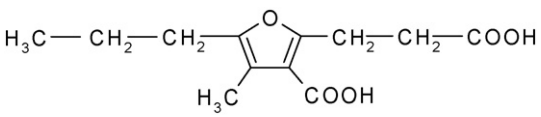
Niwa et al. [13] quantified indoxyl-β-D-glucuronide in uremic serum and urine of uremic patients. The serum level of indoxyl-β-D-glucuronide is increased in uremic patients. The production of indoxyl- β-D-glucuronide is suppressed by the administration of the oral sorbent AST-120, and serum indoxyl- β-D-glucuronide can be efficiently removed by hemodialysis.

2.2. p-cresol sulfate

p-cresol is one of the most extensively studied uremic toxins, which has been shown to be toxic in vitro. Recently, however, de Loor et al. [14] demonstrated using GC/MS that most *p*-cresol in human is present as its sulfated metabolite, and a small proportion is glucuronidated. Unconjugated *p*-cresol is undetectably low in most cases. Martinez et al. [15] also found by using HPLC and MS that *p*-cresol sulfate (Fig. 2) was accumulated in the plasma from hemodialysis patients, but no detectable unconjugated *p*-cresol. *p*-cresol sulfate and another protein-bound uremic toxin, indoxyl sulfate, were 94% and 93% bound to albumin, respectively. Thus, *p*-cresol circulates in the form of its sulfate conjugate, *p*-cresol sulfate. *p*-cresol sulfate is poorly removed by hemodialysis because its clearance is limited by albumin binding. *p*-cresol sulfate shows a pro-inflammatory effect on leucocytes [16].

2.3. 3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid

Niwa et al. identified 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) (Fig. 3) in uremic serum using GC/MS [17] and LC/MS [18]. The serum concentration of CMPF is increased in hemodialysis patients, and it cannot be removed by conventional hemodialysis due to its strong binding to serum albumin. CMPF causes a number of problems in uremic patients. CMPF has been proposed to be involved in inhibition of drug binding to albumin, anemia, thyroid dysfunction, inhibition of tubular secretion, neurological symptoms and inhibition of drug metabolism in the liver. CMPF can directly inhibit the uptake of erythromycin by inhibiting Oatp2, a hepatic uptake and/or efflux transporter [19].



3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid
(MW 240)

Fig. 3. Chemical structure of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid.