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☆

Toshimitsu Niwa*

Nagoya University Hospital, Department of Clinical Preventive Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8560, Japan

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.

Contents

1. Introduction

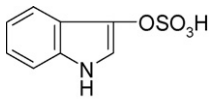
This article reviews recent papers, particularly published after my previous review [1], on the use of mass spectrometry (MS) for the analysis of uremic toxins. The uremic syndrome is considered to be caused by an accumulation of uremic toxins due to kidney dysfunction. Ninety compounds have been considered 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.

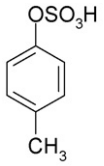
* Tel.: +81 52 744 1980; fax: +81 52 744 1954.
E-mail address: tniwa@med.nagoya-u.ac.jp.

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
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.

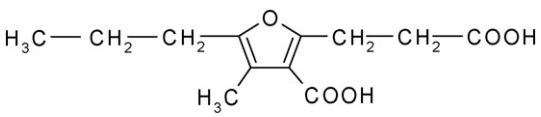
Niwa et al. [13] quantified indoxyl-β-D-glucuronide in uremic serum and urine of uremic patients. The serum level of indoxyl-β-D-glucuronide is increased in uremic patients. The production of indoxyl- β-D-glucuronide is suppressed by the administration of the oral sorbent AST-120, and serum indoxyl-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

Sassa et al. [20] measured the serum and hair concentration of CMPF in uremic patients on hemodialysis using GC/MS. The serum concentration of CMPF was increased in uremic patients. However, the hair concentration of CMPF was low in the uremic patients as compared with the healthy subjects. Since CMPF was measurable in the sweat collected from healthy subjects, the contribution of sweat to the measurement of CMPF in hair was considerable. The fact that the uremic patients undergoing hemodialysis therapy had less sweat than healthy subjects, may explain the lower concentration of CMPF in the patients' hair.

Huang et al. [21] reported a sensitive and specific LC/MS/MS method to measure CMPF level in human plasma. The average concentration in uremic patients was about 10 times higher than that in healthy controls.

2.4. Asymmetric dimethylarginine

Asymmetric dimethylarginine (ADMA) (Fig. 4) and symmetric dimethylarginine (SDMA) are naturally occurring analogues of L-arginine, a substrate for the enzyme nitric oxide synthase (NOS), which produces NO. ADMA is a competitive inhibitor of NOS, whereas SDMA has virtually no inhibitory effect on NOS activity, but shares the pathway for cell entry and transport with L-arginine and ADMA. ADMA is not only a uremic toxin, but also a marker of endothelial dysfunction and cardiovascular disease.

ADMA and SDMA are produced in posttranslational methylation of proteins in the cytoplasm of all human cells, catalyzed by S-adenosylmethionine protein N-methyltransferases (protein methylases) using S-adenosylmethionine as the methyl group donor. In the course of protein turnover, ADMA and SDMA are liberated into blood. ADMA is renally excreted into urine, or converted by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) to produce dimethylamine and citrulline. The enzyme activity of DDAH is inhibited by NO and oxidative stress. For SDMA, only renal excretion has been observed. In patients with chronic kidney disease, ADMA and SDMA accumulate in the plasma due to renal dysfunction.

Endothelial dysfunction and damage are systemic processes that play a central role in the pathogenesis of hypertension and atherosclerotic cardiovascular disease. Uremia is associated with impaired endothelium-dependent vasodilatation that may be partly a consequence of increased circulating levels of ADMA. Martens-Lobenhoffer and Bode-Böger [22] reviewed chromatographic–mass spectrometric methods for the quantification of ADMA, SDMA and L-arginine in biological fluids.

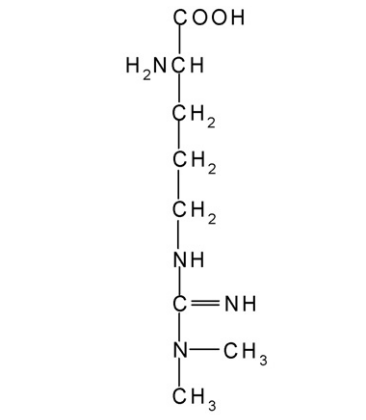


Fig. 4. Chemical structure of asymmetric dimethylarginine.

MacAllister et al. [23] demonstrated using HPLC that there is a 3-fold increase in plasma ADMA concentration in uremic patients compared to controls, and that SDMA accumulates to a 8-fold increase. Vishwanathan et al. [24] developed a LC/MS/MS method to extract and quantitate ADMA, SDMA, N-monomethyl L-arginine and L-arginine from human plasma. ^{13}C6-L-arginine was used as the internal standard for the assay. Protein precipitation using acetonitrile gave good recoveries of all the compounds from plasma. Tsikas et al. [25] developed a GC/MS/MS method for the accurate quantification of ADMA in human plasma or serum and urine using ^{2}H3-methyl ester ADMA as the internal standard. ADMA was converted to its methyl ester pentafluoropropionic anhydride derivative. Albsmeier et al. [26] developed a GC/MS method for the quantification of ADMA in cell culture supernatants and in small volumes of plasma. ADMA was concentrated by solid phase extraction and converted to its methyl ester pentafluoropropionic amide derivative. ^{2}H6-ADMA was used as the internal standard. Huang et al. [27] developed a LC/MS method coupled with an atmospheric pressure chemical ionization (APCI) interface for simultaneous separation and determination of ADMA, SDMA, N-monomethyl-L-arginine and L-arginine in human plasma. Sample pretreatment is not required other than deproteinization with 5-sulfosalicylic acid.

D'Apolito et al. [28] developed and validated a hydrophilic interaction chromatographic method coupled to tandem MS (HILIC-MS/MS) for separation and simultaneous quantification of ADMA, SDMA, and L-arginine, with a short run time (less than 5 min) using a small volume of human plasma (0.02 mL).

2.5. Homocysteine

Hyperhomocysteinemia has been reported to be an important risk factor for the development of atherosclerosis. Serum concentration of homocysteine (Fig. 5) has been measured by GC/MS and LC/MS/MS. Bachmann et al. [29] measured serum concentration of homocysteine by GC/MS, and reported that hyperhomocysteinemia is an independent risk factor for vascular disease in hemodialysis patients. Henning et al. [30] also measured serum concentration of homocysteine, and found that in the hemodialysis patients receiving oral vitamin supplementation (vitamin B6, vitamin B12, and folic acid), serum homocysteine concentrations were significantly lower than that in hemodialysis patients not receiving the vitamins. Pavarino-Bertelli et al. [31] measured plasma concentration of homocysteine by LC/MS/MS, and demonstrated that hyperhomocysteinemia and methylene-tetrahydrofolate reductase enzymes C677T and A1298C polymorphisms are associated with chronic allograft nephropathy in renal transplant recipients.

2.6. Dicarbonyl compounds

Glyoxal, methylglyoxal and 3-deoxyglucosone (Fig. 6) are reactive dicarbonyl intermediates in advanced Maillard reaction,

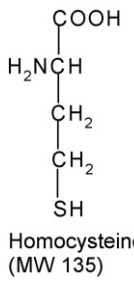


Fig. 5. Chemical structure of homocysteine.

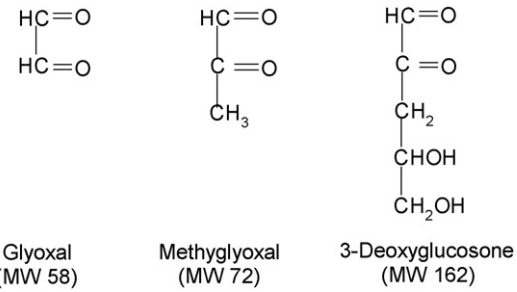


Fig. 6. Chemical structure of glyoxal, methylglyoxal and 3-deoxyglucosone.

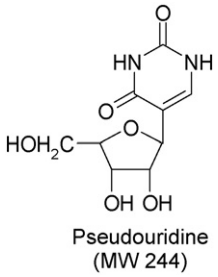


Fig. 8. Chemical structure of pseudouridine.

which form AGEs by reaction with both lysine and arginine residues in protein. 3-Deoxyglucosone rapidly reacts with protein amino groups to form AGEs such as imidazolone, pyrraline, N^{ε}-(carboxymethyl)lysine and pentosidine, among which imidazolone is the AGE most specific for 3-deoxyglucosone [32]. Niwa et al. [33] demonstrated by stable isotope dilution GC/MS that plasma 3-deoxyglucosone levels are markedly increased in uremia. Odani et al. [34] demonstrated by LC/ESI–MS that these dicarbonyl compound levels were increased in uremic plasma as compared with healthy controls.

Nakayama et al. [35] measured plasma levels of glyoxal, methylglyoxal and 3-deoxyglucosone using LC/MS in healthy controls and chronic kidney disease patients with or without diabetes. They found that plasma glyoxal and methylglyoxal levels increase as the stages of chronic kidney disease progress. However, plasma 3-deoxyglucosone levels did not differ among chronic kidney disease groups, and were significantly higher in diabetic patients than in non-diabetic patients.

2.7. Nicotinamide metabolites

Slominska et al. [36] and Rutkowski et al. [37] demonstrated by using HPLC and MS that plasma concentrations of nicotinamide metabolites such as N-methyl-2-pyridone-5-carboxamide (Met2PY) (Fig. 7) and N-methyl-4-pyridone-3-carboxamide (Met4PY) are increased in uremic patients. Met2PY inhibits poly(ADPribose)polymerase-1, which is involved in a variety of physiological events such as chromatin decondensation, DNA replication, DNA repair, regulation of gene expression, cellular differentiation and apoptosis. Concentrations of nicotinamide and its end products in tissues were measured using HPLC and MS [38]. Both Met2PY and Met4PY were significantly elevated not only in the blood but also in tissues of 5/6 nephrectomized uremic rats such as liver, lungs, skeletal muscles and the kidneys.

Carrey et al. [39] identified 2-pyridone-5-carboxamide ribonucleoside triphosphate (2PyTP) in uremic erythrocytes using liquid chromatography and MS. Plasma concentrations of Met2PY correlated with 2PyTP in the erythrocyte. Thus, Met2PY accumulates in parallel with the nucleotide 2PyTP in renal failure: either may be a uremic toxin, since both increase with the degree of renal failure. Further, Carrey et al. [40] have investigated an unusual nucleotide that accumulates, with precursors, in the erythrocytes of patients

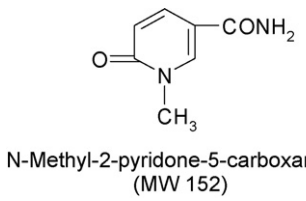


Fig. 7. Chemical structure of N-methyl-2-pyridone-5-carboxamide.

in uremia. This nucleotide is related chemically to the NAD breakdown product, Met2Py, found in high concentrations in the plasma of uremic patients. Both Met2Py and the nucleotide accumulate to high concentrations in uremic blood.

2.8. Modified nucleosides

Takeda et al. [41] characterized 20 nucleosides in serum and 23 nucleosides in urine from both healthy subjects and uremic patients by capillary liquid chromatography-frit-fast atom bombardment MS. Most of them were modified nucleosides derived from tRNA breakdown products. The endogenous ribonucleosides were retained at higher levels in uremic serum, and may play a contributory role as toxins responsible for clinical symptoms of uremia. Niwa et al. [42] reported that the serum levels of xanthosine and all modified ribonucleosides were increased in undialyzed patients with chronic renal failure, and patients undergoing hemodialysis and continuous ambulatory peritoneal dialysis (CAPD). The serum level of pseudouridine was markedly increased in all the uremic patients, especially CAPD patients. CAPD patients showed markedly increased serum levels of modified ribonucleosides such as pseudouridine (Fig. 8), 1-methylinosine, and N^2,N^2-dimethylguanosine and N^4-acetylcytidine as compared with the hemodialysis patients.

3. Middle-molecular-weight peptides

The uremic solutes ranging from 500 to 5000 Da are insufficiently eliminated by conventional hemodialysis, and may act as uremic toxins. Kaplan et al. [43] applied a new micro-preparative procedure including sodium dodecyl sulfate electrophoresis and liquid chromatography for isolation of middle molecule peptides from uremic sera. Microsequencing and MS/MS analyses of these peptides showed that most of the identified middle molecules represented the N- and C-terminal fragments of the α- and β-chains of fibrinogen.

Chu et al. [44] characterized six middle molecular compounds by multi-step chromatographic isolation and MALDI-TOF-MS. Three of these uremic middle molecular compounds are important, as they readily accumulate in sera of uremic patients, but are normally excreted in healthy subjects.

Li et al. [45] found 17 endogenous middle molecular compounds in uremic plasma and normal urine, and identified an octapeptide (1007.94 Da) with an amino acid sequence of Val-Val-Arg-Gly-Cys-Thr-Trp-Trp by using gel permeation chromatography, ion exchange chromatography, and reversed-phase HPLC and MS.

Weissinger et al. [46] detected between 500 and 1000 polypeptides ranging from 800 to 10,000 Da in ultrafiltrate from uremic plasma by using CE and MS. They identified a 950.6 Da polypeptide as a fragment of the salivary proline-rich protein, and a 1291.8 Da fragment derived from α-fibrinogen.

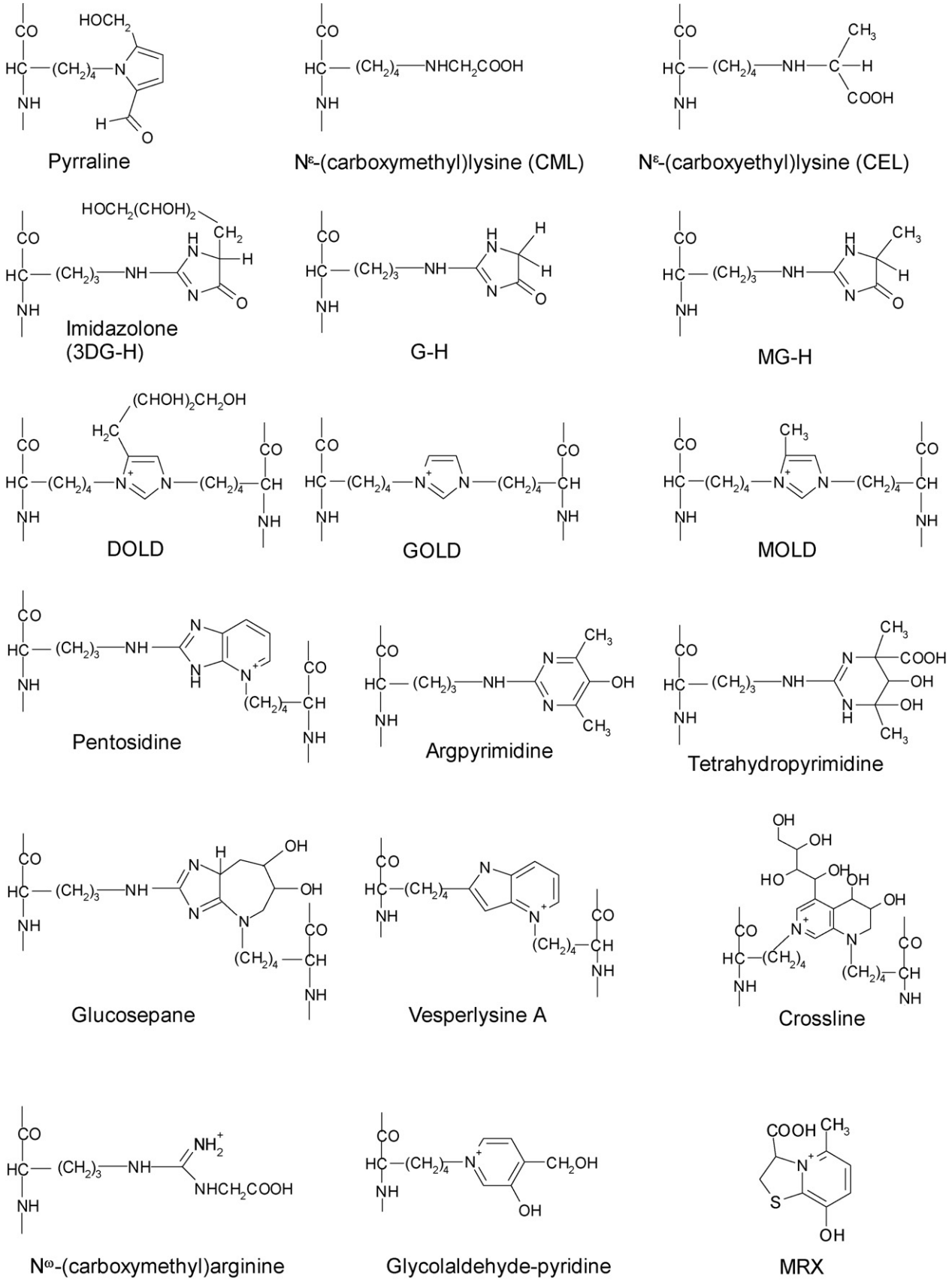


Fig. 9. Chemical structure of AGEs. 3-DG-H: 3-deoxyglucosone-derived hydroimidazolone, G-H: glyoxal-derived hydroimidazolone, MG-H: methylglyoxal-derived hydroimidazolone, DOLD: 3-deoxyglucosone-derived lysine dimmer (1,3-di(N$^{\varepsilon}$-lysino)-4-(2,3,4-trihydroxybutyl)-imidazolium salt), GOLD: glyoxal-lysine dimer (1,3-di(N$^{\varepsilon}$-lysino)imidazolium salt), MOLD: methylglyoxal-lysine dimer (1,3-di(N$^{\varepsilon}$-lysino)-4-methyl-imidazolium salt), and MRX: 8-hydroxy-5-methyldihydrothiazolo[3,2-α]pyridinium-3-carboxylate.

4. Modified proteins

4.1. Proteins modified with advanced glycation end products (AGEs)

The term protein glycation summarizes non-enzymatic reactions between amino groups of proteins and sugars or sugar degradation products, leading to early glycation products and AGEs (Fig. 9). Protein glycation is involved in the progression of not only diabetes mellitus, but also uremia. MS has been successfully used to analyze the structure of glycated proteins and peptides [47]. Kislinger et al. [48] applied MALDI-TOF-MS to the investigation of glycation processes. The analyses of plasma proteins from uremic patients allowed a precise determination of the average number of sugar residues attached to serum albumin or immunoglobulins of each patient. Further, the glycation rate of hemoglobin, isolated from diabetic blood and of β2-microglobulin (β2m) isolated from amyloid plaques of uremic patients was determined.

Thornalley et al. [49] studied molecular mass changes of serum albumin of uremic patients by MALDI-TOF-MS. They found a significant increase in the molecular mass of albumin in uremia. The molecular mass of albumin in vivo was indicative of a minimal and not a high extent of glycation.

Protein glycation adducts, early glycation adducts, such as N^{ε}-fructosyl-lysine, and AGEs are considered to be uremic toxins. Glycation adducts are found in plasma and tissue proteins (glycation adduct residues), in peptides (glycation adduct peptide residues), and glycated amino acids (glycation free adducts). The latter two analyte groups arise from proteolysis of glycated proteins and glycation of peptides and amino acids. Thornalley et al. [50] measured the concentrations of the early glycation adduct fructosyl-lysine and 12 AGEs by LC/MS/MS. Underivatized analytes were detected free in physiological fluids and in enzymic hydrolysates of cellular and extracellular proteins. Hydroimidazolones were the most important glycation biomarkers quantitatively; monolysyl adducts such as N^{ε}-(carboxymethyl)lysine and N^{ε}-(carboxyethyl)lysine, were found in moderate amounts, and bis(lysyl)imidazolium cross-links and pentosidine in lowest amounts. In uremia, the concentrations of plasma protein AGEs residues increased one- to seven-fold and free adduct concentrations increased up to 50-fold. Increased levels of these AGEs were associated with vascular complications in uremia. Further, Thornalley [51] demonstrated by LC/MS/MS that glycation free adducts accumulate markedly in the plasma of uremic patients, and are eliminated in the peritoneal dialysate. Thus, LC/MS/MS has been used to quantify concentrations, extents of protein modification, clearances, and excretion rates of glycation adducts in uremia.

Mironova and Niwa [52] characterized the molecular heterogeneity and AGEs modification of β2m extracted from the amyloid tissue of a hemodialysis patient, by using LC/ESI–MS, Western blotting and N-terminal amino acid sequence analysis. Amyloid β2m was composed of full-length β2m, truncated β2m and dimer β2m. Truncated β2m and dimer β2m were modified with AGEs such as imidazolone and N^{ε}-(carboxymethyl)lysine, and showed fluorescence characteristic of AGEs. Truncated β2m species were formed by cleavage between amino acid residues of Pro6/Ile7, Gln8/Val9 and Val9/Tyr10. Heterogeneous dimer β2m species showed the molecular masses of 22,591 and 22,675, which resulted from cross-linking between truncated β2m.

Konings et al. [53] investigated whether the use of polyglucose solution (icodextrin) for the long dwell in peritoneal dialysis patients would result in a reduction in plasma and dialysate levels of N^{ε}-(carboxymethyl)lysine, one of AGEs. N^{ε}-(Carboxymethyl)lysine was measured by stable isotope dilution MS/MS. N^{ε}-(Carboxymethyl)lysine levels in plasma increased significantly in patients treated with icodextrin, and the same held true for N^{ε}-(carboxymethyl)lysine levels in dialysate. Thus, contrary to expectation, plasma and dialysate levels of N^{ε}-(carboxymethyl)lysine increased in patients treated using icodextrin for the long dwell.

4.2. Oxidized albumin

Musante et al. [54,55] demonstrated using LC/MS/MS that plasma albumin undergoes massive oxidation in patients with focal segmental glomerulosclerosis, involving stable sulphonation $SO3^{-}$ of the free SH of Cys 34 with +48 Da increase in exact mass of the protein and formation of a fast moving isoform in the pH range between 5 and 7. This is the first demonstration of massive oxidation of albumin in vivo that reflects a functional role of the protein. Thus, free radicals should be implicated in the pathogenesis of proteinuria in patients with focal segmental glomerulosclerosis.

Recently, Bruschi, et al. [56] demonstrated using LC/ESI–MS/MS that albumin in hemodialysis patients presented only intermediate oxidation products such as sulfenic (SO2), sulfonic (SO) and methionine sulfoxide (C5H9NO2S) involving Cys 165–269 and Met 329–548, but did not present $SO3^{-}$ at Cys 34. Thus, the oxido-redox status of plasma albumin in hemodialysis patients lacks the hallmarks of the advanced oxidation products.

5. Conclusion

Recently, the research on uremic toxins arouses interest, owing to the new analytical methodologies of MS. MS has become a prerequisite method for metabolome and proteome analysis of uremic toxins accumulated in uremic serum. Knowledge on the clinical role of uremic toxins in uremic syndrome has been expanding. Some uremic toxins such as indoxyl sulfate and *p*-cresol sulfate are derived from metabolites produced by intestinal bacteria, and their removal by oral sorbent improves some uremic syndrome (progression of chronic kidney disease and cardiovascular disease). The role of uremic toxins in the pathogenesis of cardiovascular disease in chronic kidney disease patients is one of main recent topics in the field of uremic toxicity. Further research to specifically remove the toxins responsible for cardiovascular disease is required. As a future trend, the use of imaging MS enables the direct analysis of uremic toxins accumulated in the tissues of chronic kidney disease patients to study their role in clinical symptoms.

References

[1] T. Niwa, Mass Spectrom. Rev. 16 (1997) 307.
[2] A. Yavuz, C. Tetta, F.F. Ersoy, V. D'intini, R. Ratanarat, M. De Cal, M. Bonello, V. Bordoni, G. Salvatori, E. Andrikos, G. Yakupoglu, N.W. Levin, C. Ronco, Semin. Dial. 18 (2005) 203.
[3] A.B. Kanu, P. Dwivedi, M. Tam, L. Matz, H.H. Hill Jr., J. Mass Spectrom. 43 (2008) 1.
[4] R.G. Cooks, Z. Ouyang, Z. Takats, J.M. Wiseman, Science 311 (2006) 1566.
[5] K.D. Herring, S.R. Oppenheimer, R.M. Caprioli, Semin. Nephrol. 27 (2007) 597.
[6] T. Niwa, N. Takeda, A. Tatematsu, K. Maeda, Clin. Chem. 34 (1988) 2264.
[7] N. Takeda, T. Niwa, K. Maeda, M. Shibata, A. Tatematsu, J. Chromatogr. 431 (1988) 418.
[8] T. Niwa, M. Ise, J. Lab. Clin. Med. 124 (1994) 96.
[9] T. Niwa, M. Ise, T. Miyazaki, Am. J. Nephrol. 14 (1994) 207.
[10] A. Adijiang, S. Goto, S. Uramoto, F. Nishijima, T. Niwa, Nephrol. Dial. Transplant. 23 (2008) 1892.
[11] G. Muteliefu, A. Enomoto, T. Niwa, J. Renal Nutr., in press.
[12] Z. Tumur, T. Niwa, Am. J. Nephrol., in press.
[13] T. Niwa, T. Miyazaki, S. Tsukushi, K. Maeda, Y. Tsubakihara, A. Owada, T. Shiigai, Nephron 74 (1996) 72.
[14] H. de Loor, B. Bammens, P. Evenepoel, V. De Preter, K. Verbeke, Clin. Chem. 51 (2005) 1535.
[15] A.W. Martinez, N.S. Recht, T.H. Hostetter, T.W. Meyer, J. Am. Soc. Nephrol. 16 (2005) 3430.
[16] E. Schepers, N. Meert, G. Glorieux, J. Goeman, J. Van der Eycken, R. Vanholder, Nephrol. Dial. Transplant. 22 (2007) 592.
[17] T. Niwa, N. Takeda, K. Maeda, M. Shibata, A. Tatematsu, Clin. Chim. Acta 173 (1988) 127.

[18] T. Niwa, I. Kawagishi, N. Ohya, Clin. Chim. Acta 226 (1994) 89.
[19] H. Sun, Y. Huang, L. Frassetto, L.Z. Benet, Drug Metab. Dispos. 32 (2004) 1239.
[20] T. Sassa, H. Matsuno, M. Niwa, O. Kozawa, N. Takeda, T. Niwa, T. Kumada, T. Uematsu, Arch. Toxicol. 73 (2000) 649.
[21] Y. Huang, H. Sun, L. Frassetto, L.Z. Benet, E.T. Lin, Rapid Commun. Mass Spectrom. 20 (2006) 1611.
[22] J. Martens-Lobenhoffer, S.M. Bode-Böger, J. Chromatogr. B. 851 (2007) 30.
[23] R.J. MacAllister, M.H. Rambausek, P. Vallance, D. Williams, K.H. Hoffmann, E. Ritz, Nephrol. Dial. Transplant. 11 (1996) 2449.
[24] K. Vishwanathan, R.L. Tackett, J.T. Stewart, M.G. Bartlett, J. Chromatogr. B 748 (2000) 157.
[25] D. Tsikas, B. Schubert, F.M. Gutzki, J. Sandmann, J.C. Frölich, J. Chromatogr. B 798 (2003) 87.
[26] J. Albsmeier, E. Scwedhelm, F. Schulze, M. Kastner, R.H. Böger, J. Chromatogr. B 809 (2004) 59.
[27] L.F. Huang, F.Q. Guo, Y.Z. Liang, B.Y. Li, B.M. Cheng, Anal. Bioanal. Chem. 380 (2004) 643.
[28] O. D'Apolito, G. Paglia, F. Tricarico, D. Garofalo, A. Pilotti, O. Lamacchia, M. Cignarelli, G. Corso, Clin. Biochem. 41 (2008) 1391.
[29] J. Bachmann, M. Tepel, H. Raidt, R. Riezler, U. Graefe, K. Langer, W. Zidek, J. Am. Soc. Nephrol. 6 (1995) 121.
[30] B.F. Henning, R. Riezler, M. Tepel, K. Langer, H. Raidt, U. Graefe, W. Zidek, Nephron 83 (1999) 314.
[31] E.C. Pavarino-Bertelli, M.P. Sanches de Alvarenga, E.M. Goloni-Bertollo, M.A. Baptista, R. Haddad, N.F. Hoerh, M.N. Eberlin, M. Abbud-Filho, Transplant. Proc. 36 (2004) 2979.
[32] T. Niwa, J. Chromatogr. B 731 (1999) 23.
[33] T. Niwa, T. Katsuzaki, T. Momoi, T. Miyazaki, H. Ogawa, A. Saito, S. Miyazaki, K. Maeda, N. Tatemichi, Y. Takei, Kidney Int. 49 (1996) 861.
[34] H. Odani, T. Shinzato, Y. Matsumoto, J. Usami, K. Maeda, Biochem. Biophys. Res. Commun. 256 (1999) 89.
[35] K. Nakayama, M. Nakayama, M. Iwabuchi, H. Terawaki, T. Sato, M. Kohno, S. Ito, Am. J. Nephrol. 28 (2008) 871.
[36] E.M. Slominska, R.T. Smolenski, M. Szolkiewicz, N. Leaver, B. Rutkowski, H.A. Simmonds, J. Swierczynski, Mol. Cell. Biochem. 231 (2002) 83.
[37] B. Rutkowski, E. Slominska, M. Szolkiewicz, R.T. Smolenski, C. Striley, P. Rutkowski, J. Swierczynski, Kidney Int. Suppl. 84 (2003) S19.
[38] P. Rutkowski, E.M. Slominska, W. Wołyniec, R.T. Smoleński, M. Szolkiewicz, J. Swierczyński, B. Rutkowski, J. Renal Nutr. 18 (2008) 56.
[39] E.A. Carrey, R.T. Smolenski, S.M. Edbury, A. Laurence, A.M. Marinaki, J.A. Duley, L. Zhu, D.J. Goldsmith, H.A. Simmonds, Clin. Chim. Acta 335 (2003) 117.
[40] E.A. Carrey, R.T. Smolenski, S.M. Edbury, A. Laurence, A.M. Marinaki, J.A. Duley, L.M. Zhu, D.J. Goldsmith, H.A. Simmonds, Nucleosides Nucleotides Nucleic Acids 23 (2004) 1135.
[41] N. Takeda, H. Yoshizumi, T. Niwa, J. Chromatogr. B 746 (2000) 51.
[42] T. Niwa, N. Takeda, H. Yoshizumi, Kidney Int. 53 (1998) 1801.
[43] B. Kaplan, M. Cojocaru, E. Unsworth, A. Knecht, B.M. Martin, J. Chromatogr. B 796 (2003) 141.
[44] J. Chu, Z. Yuan, X. Liu, Q. Wu, H. Mi, B. He, Clin. Chim. Acta 311 (2001) 95.
[45] G. Li, J. Chu, X. Liu, Z. Yuan, Clin. Chim. Acta 350 (2004) 89.
[46] E.M. Weissinger, T. Kaiser, N. Meert, R. De Smet, M. Walden, H. Mischak, R.C. Vanholder, Nephrol. Dial. Transplant. 19 (2004) 3068.
[47] T. Niwa, Mass Spectrom. Rev. 25 (2006) 713.
[48] T. Kislinger, A. Humeny, M. Pischetsrieder, Curr. Med. Chem. 11 (2004) 2185.
[49] P.J. Thornalley, M. Argirova, N. Ahmed, V.M. Mann, O. Argirov, A. Dawnay, Kidney Int. 58 (2000) 2228.
[50] P.J. Thornalley, S. Battah, N. Ahmed, N. Karachalias, S. Agalou, R. Babaei-Jadidi, A. Dawnay, Biochem. J. 375 (2003) 581.
[51] P.J. Thornalley, Perit. Dial. Int. 25 (2005) 522.
[52] R. Mironova, T. Niwa, J. Chromatogr. B 758 (2001) 109.
[53] C.J. Konings, C.G. Schalkwijk, F.M. van der Sande, K.M. Leunissen, J.P. Kooman, Perit. Dial. Int. 25 (2005) 591.
[54] L. Musante, M. Bruschi, G. Candiano, A. Petretto, N. Dimasi, P. Del Boccio, A. Urbani, G. Rialdi, G.M. Ghiggeri, Biochem. Biophys. Res. Commun. 349 (2006) 668.
[55] L. Musante, G. Candiano, A. Petretto, M. Bruschi, N. Dimasi, G. Caridi, B. Pavone, P. Del Boccio, M. Galliano, A. Urbani, F. Scolari, F. Vincenti, G.M. Ghiggeri, J. Am. Soc. Nephrol. 18 (2007) 799.
[56] M. Bruschi, A. Petretto, G. Candiano, L. Musante, E. Movilli, L. Santucci, A. Urbani, R. Gusmano, E. Verrina, G. Cancarini, F. Scolari, G.M. Ghiggeri, J. Chromatogr. B 864 (2008) 29.