The application of MALDI-TOF for diagnostics of mucopolysaccharidoses and mucolipidoses

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Abstract

The application of MALDI-TOF for diagnostics of mucopolysaccharidoses and mucolipidoses Mucopolysaccharidoses (MPSs) and mucolipidoses (MLs) are part of inherited metabolic disorders also known as lysosomal storage diseases. These diseases affect function of many vital organs (heart, lungs, spleen) and central nervous system. Usual approaches towards diagnostics of these disorders are enzymatic assay genetic analysis or other methods (thin-layer chromatography, capillary electrophoresis etc.). In this work, matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis of permethylated urine samples from patients with four different types of MPSs and MLs was used for purpose of their diagnostics.

Keywords: *MALDI-TOF; lysosomal storage diseases; mucopolysaccharidosis; mucolipidosis*

Introduction and Objectives

Mucopolysaccharidoses (MPSs) and mucolipidoses (MLs) are lysosomal storage diseases (LSDs), a group of inherited metabolic disorders caused by enzyme deficiencies resulting in accumulation of undegraded saccharide substrate. This process affects central nervous system and can lead to a broad spectrum of clinical manifestations such as mental retardation, development delay or hydrocephalus. LSDs affect other organs as well (heart, spleen, liver etc.). Mucopolysaccharidoses include eight individual disorders (denoted with Roman numerals) and each of these disorders has a wide spectrum of phenotypic variation depending on the specific mutation. Mucolipidoses are classified into four types: ML I - IV [1 – 3]. MPSs and MLs are usually diagnosed by enzymatic assay, genetic analysis, or other methods, such as thin-layer chromatography (TLC), dimethyl methylene blue (DMB) assay or capillary electrophoresis [4]. LSDs can be successfully diagnosed also by the application of spectral methods such as nuclear magnetic resonance (NMR), electrospray ionisation mass spectroemtry (ESI-MS) and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). These analyses have many advantages, e.g. MALDI-TOF analysis requires small sample volume and provides possibility of automatization of the

analytical process. Therefore this method is suitable for the diagnostics of lysosomal storage disorders [5].

In this work we performed MALDI-TOF analysis of permethylated urine samples obtained from patients with four different LSD types: MPS I (female, 11 y.o.), MPS II (male, 15 y.o.), MPS IIIA (male, 6 y.o.) and ML II (male, 2 y.o.) with the goal aimed to identify the oligosaccharide biomarkers of these disorders. As a negative control, permethylated urine sample of healthy specimen was used (male, 6 y.o.). The main reason for analysis of urine samples is a non-invasive character of this sample type.

Materials and methods

First step of urine sample preparation was dissolution of 50 μL of each sample in water (LC-MS quality). Samples were then mixed vigorously on vortex and then frozen at – 80 °C. After freezing, the samples were dried on lyophilizator overnight and then permethylated. Permethylation process started with addition of DMSO into the NaOH and mixing into a slurry. Approximately 150 μL of this slurry was added to each sample and then 150 μL of iodomethane was added into the mixture and mixed vigorously on vortex. Mixed samples were incubated on a shaker (2000 rpm, 50 min, 25 °C). Mixed samples were then extracted with chloroform and washed with ice-cold water until the reach of neutral pH value. Samples were then dried at laboratory temperature. Permethylated samples were then dissolved in 10 μL of water/methanol solution (50/50).

1 μL of permethylated sample was spotted onto a MALDI plate with 1 μL of Na-DHB matrix (2,5-dihydroxybenzoic acid dissolved in TA30 with the addition 1 mM NaOH) and dried at laboratory temperature. MALDI-TOF analyses were performed on MALDI mass spectrometer *UltrafleXtreme II (Bruker Daltonics, Germany)* in positive reflectron ionisation mode. The results were evaluated with software programs *flexAnalysis 3.4 (Bruker Daltonics, Germany)* and *GlycoWorkbench (www.eurocarbdb.org)*.

Results and discussion

Primary goal of this work was to obtain potential biomarkers for four representative types of LSDs in permethylated urine samples using MALDI-TOF mass spectrometry. As a negative control, permethylated urine sample of a healthy specimen was analysed (Fig. 1.). For MPS I, nine signals of potential biomarkers for this disease were identified (Fig. 2.). The most intensive identified signal of this spectrum, *m/z* 2546.9 Da, corresponds to the $Neu_5Ac_2Hex_5HexNAc$ structure.

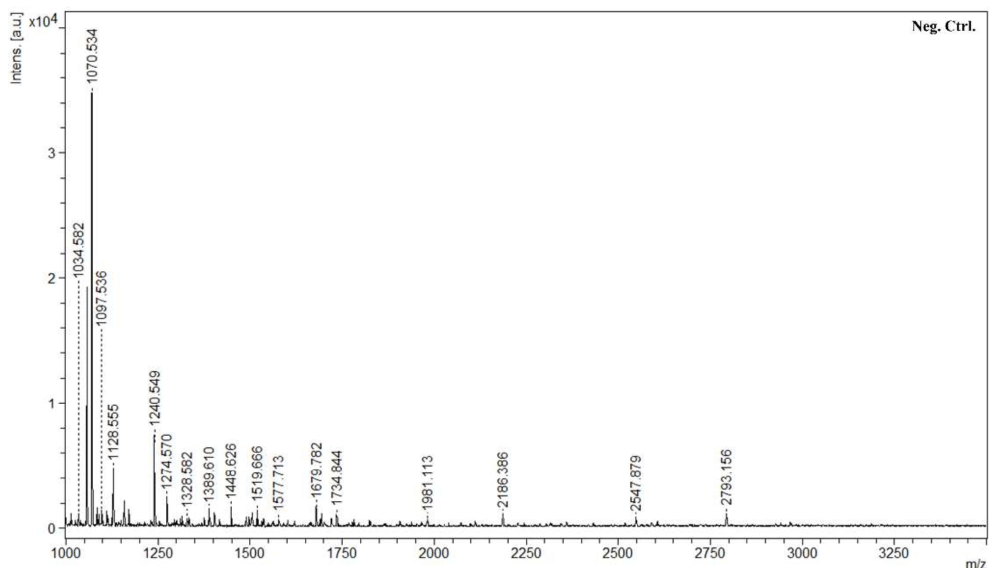

Fig. 1. MALDI-TOF spectrum of permethylated urine sample of a healthy specimen

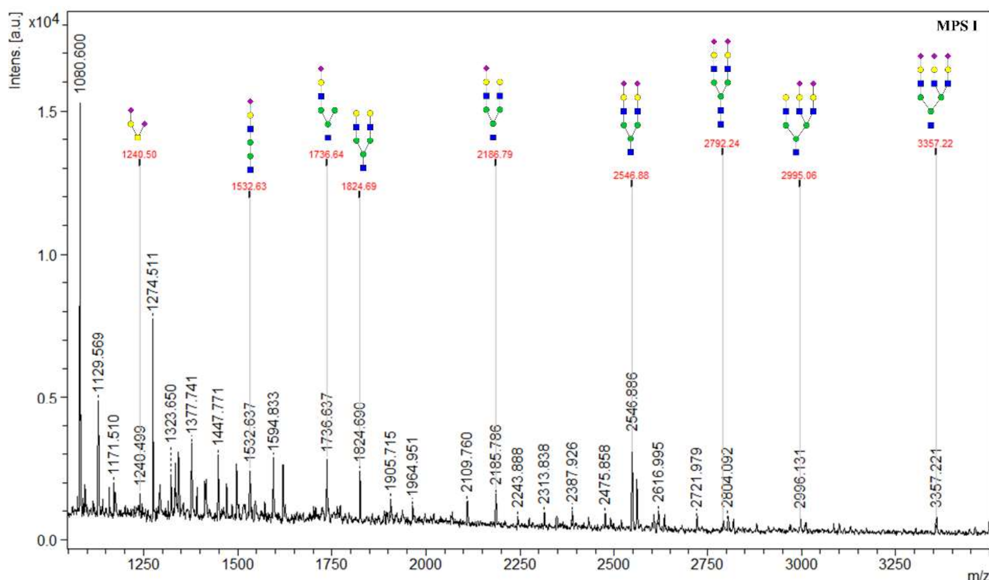

Fig. 2. MALDI-TOF spectrum of permethylated urine sample of MPS I patient

Using MALDI-TOF analysis, potential biomarkers for MPS II were identified as well. In this case, we identified signals of six biomarkers for this disease and the most intense signal corresponds with the $Neu_5Ac_1Hex_3HexNAc_2$ structure (*m/z* 1532.6 Da) (Fig. 3.). For MPS IIIA, six potential biomarkers were identified. The most intense signal corresponds to $Neu_5Ac_2Hex_1HexNAc$ (*m/z* 1240.6 Da) (Fig. 4.). The last urine sample we analysed using MALDI-TOF analysis came from the patient with ML II (Fig. 5.). In this case, we identified eleven different oligosaccharide signals that represent potential biomarkers for this disorder. The most intense signal corresponds to $Neu_5Ac_1Hex_3HexNAc_2$ structure (*m/z* 1532.6 Da).

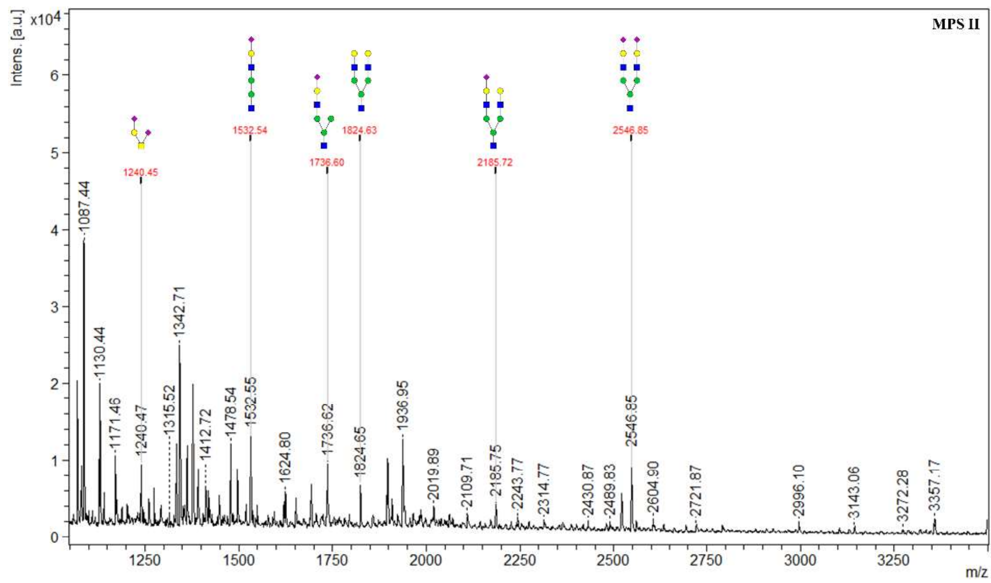

Fig. 3. MALDI-TOF spectrum of permethylated urine sample of MPS II patient

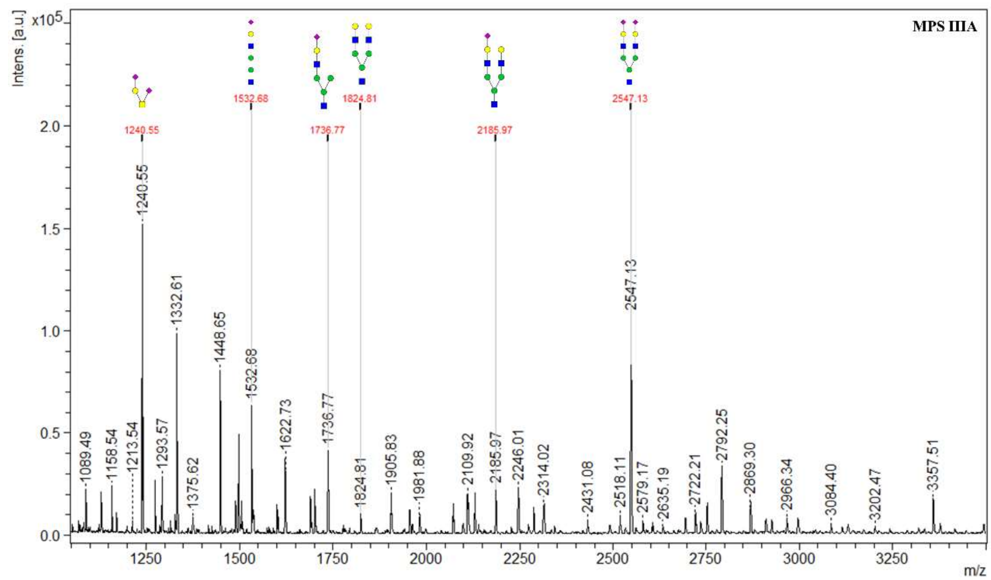

Fig. 4. MALDI-TOF spectrum of permethylated urine sample of MPS IIIA patient

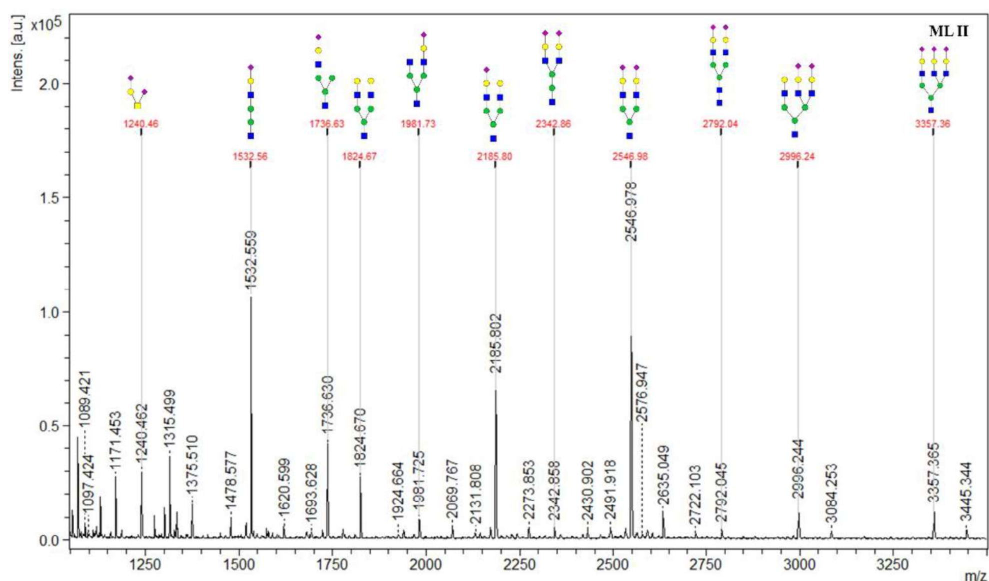

Fig. 5. MALDI-TOF spectrum of permethylated urine sample of ML II patient

We can see that some signals present in the negative control are present in the spectra of LSDs as well. However, intensities of these signals are significantly lower in case of negative control, than in case of patient samples. Also, many signals correspond to different LSDs as common biomarkers. Some of these signals are shown in Fig. 2 – 5, e.g. signal *m/z* 1532.3 Da, which is published in literature as a biomarker for ML II, but also for other types of LSDs, such as Galactosialidosis or Sialidosis [6]. We could see these similarities especially in case of spectra of urine samples obtained from patients with MPS II and MPS IIIA, where we identified the same signals for both diseases. However, there are great differences in the intensities of these signals, e.g. the signal of $Neu_5Ac_2Hex_1HexNAc$ structure (*m/z* 1240.6 Da) has a significantly higher intensity in the spectrum of urine sample from MPS IIIA patient (Fig. 4). In spectrum of urine sample obtained from patient with MPS II (Fig. 3) has higher intensity signal corresponding with $Hex_5HexNAc_3$ structure (*m/z* 1824.6 Da). Differences in signal intensities and diversity of spectra showcase that every disease has a different ”fingerprint“ leading to diagnostics of this diseases.

Conclusions

In this work, we analysed permethylated urine samples of four patients with different LSDs: MPS I (female, 11 y.o.), MPS II (male, 15 y.o.), MPS IIIA (male, 6 y.o.) and ML II (male 2 y.o.). The main goal was to identify signals of potential biomarkers of these diseases using MALDI-TOF mass spectrometry. Using this method, we successfully identified signals of oligosaccharide biomarkers of these diseases. Despite the fact that some of these signals

were common for these diseases and thus they do not serve as a specific biomarkers, their intensities and number in each disease were different. This overall “fingerprint” could be considered as a specific for every disease. Unfortunately, due to the rarity of these disorders, it was not possible to obtain sufficient number of samples for every disorder and to obtain statistically significant results confirming this conclusion.

Acknowledgments

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