CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 44, Issue 5, May 2016
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Chin J Anal Chem, 2016, 44(5), 716–722.

RESEARCH PAPER

Analysis of Urine Peptidome and Post-translation Modifications by Nano Liquid Chromatography-High Resolution Tandem Mass Spectrometry

HONG Xiao-Yu[1], LI Shui-Ming[2], WANG Yong[2,*]

[1] College of Life and Ocean Science, Shenzhen Key Laboratory of Microbial Genetic Engineering, Shenzhen University, Shenzhen 518060, China

[2] College of Life and Ocean Science, Shenzhen Key Laboratory of Marine Bioresources and Ecology, Shenzhen University, Shenzhen 518060, China

Abstract: Proteomics is an emerging field branching from proteomics that targets endogenous peptides of a whole organism or a subsystem. Many peptides in body fluids including urine are biomarkers with higher clinical sensitivity and specificity. In this study, we separated and enriched these peptides in human urine by $LaPO_4$-graphene oxide composite nanomaterial, then identified and analyzed them by nano liquid chromatography-high resolution tandem mass spectrometry. In single urine sample, we identified 790 peptides which belonged to 123 proteins and some post-translation modifications including oxidation, phosphorylation, deamidated etc. There existed a series of peptide ladders in urine peptidomic. Finally, the string analysis at protein level revealed strong interaction among these proteins to which the identified peptides belong. This method can provide support for finding the biomarkers of disease in urine.

Key Words: Peptidome; High resolution tandem mass spectrometry; Urine; Post-translation modifications

1 Introduction

With the rapid development of genomics, transcriptomics and proteomics in the field of life sciences[1], the peptidomics gradually enter into people's horizons. Peptides exist in most of life body including the simplest cnidarians and the highest vertebrates[2]. Peptidomics research all endogenous peptides in body fluids, tissues and cells in vivo and their function and variation[3]. Peptides consist of amino acids and their molecular weights are generally less than 15 kDa. Some peptides may reflect abnormal metabolic processes because they are the products of protein synthesis, processing and degradation[4]. Protein may also be cut into different fragment which play particular functions[5]. Some peptides as biochemical messenger molecules, like peptide hormones, neuropeptides, cytokines, enzyme inhibitors, etc., regulate many physiological and biochemical processes. Thus peptides have a close relationship with disease. As an extension of proteomics, peptidomic can monitor the changes of the low molecular weight proteins commendably.

Peptidomic is mainly applied in screening biomarkers of disease and disease surveillance, prevention and efficacy evaluation[6]. According to the metabolism terminal, urine is so sustainable and non-invasive[7] and appropriate for detecting disease. Surface-enhanced laser desorption/ ionization time of flight mass spectrometer (SELDI-TOF MS), matrix assisted laser desorption ionization mass spectrometry (MAIDI-TOF MS or MAIDI-TOF/TOF MS)[8] and ESI^- tandem mass spectrometer, such as a triple quadrupole mass spectrometer, ion trap or quadrupole time of flight mass spectrometer[9] can be used to detect and analyze peptidome. Some researchers used MAIDI-TOF MS to find some

Received 14 October 2015; accepted 5 January 2016
*Corresponding author. Email: wyong@szu.edu.cn
This work was supported by the National Natural Science Foundation of China (No. 31470804), and the Key Program of Shenzhen, China (No. JSGG20140703163838793).

DOI: 10.1016/S1872-2040(16)60931-3

biomarker (peptides) in the early diagnosis of gastric cancer, breast cancer[10], bladder cancer[11], and IgA nephropathy[12]. But this method focuses on comparing the different peaks in identifying the differential peptidomic profiles of diseases, thus it cannot used for the proteins accurate identifying and the post-translational modification.

$LaPO_4$-graphene oxide composite nanomaterial (LaGM) was triple composites of graphene, $LaPO_4$ nanorods and Fe_3O_4 nanoparticles, which could rapidly enrich low abundance peptides, for instance, effective enrichment of peptides in urine[13]. Herein, LaGM was used to separate and enrich peptides. Besides, nano-liter high-resolution liquid chromatography tandem time of flight mass spectrometry (Nano LC-TripleTOF™ 5600) was used to identify peptides sequence and post-translational modifications. In 1.5 mL of urine from a single sample, we identified 790 peptides that belonged to 123 proteins and 91 peptides of them were oxidated, deaminated and phosphorylated. This method provided a fast and efficient analysis method for disease peptide biomarkers in urine.

2 Experimental

2.1 Instruments and reagents

The following instruments were used in this study: Eksigent nanoLC-Ultra™ 2D-LC(AB SCIEX, USA), TripleTOF 5600 high resolution mass spectrometer (AB SCIEX, USA), Protein Pilot 4.5 software (AB SCIEX, USA), vacuum freeze-drying machine (Thermo savant, USA), Ultra-pure Water Purifier (Thermo scientific, USA), C_{18} reverse phase chromatography trapping column (30 mm × 100 μm, 3 μm, 150 Å, Eksigent, USA), and C_{18} reverse phase chromatography column (150 mm × 75 μm, 3 μm, 120 Å, Eksigent, United States).

The materials and reagents used in this experiment were as follows: graphene oxide-lanthanum phosphate nano-magnetic composites (LaGM), RP mobile phase A (98% water, 0.1% formic acid and 2% acetonitrile), and RP mobile phase B (2% water, 0.1% formic acid, 98% acetonitrile).

2.2 Peptides separation and enrichment

Urine sample was collected from healthy human and stored at –80 °C after packing. Approximately 1.5 mL of urine sample was taken for peptides separation and enrichment. The urine sample was centrifuged for 10 min at 10000 rpm for removing impurities, then 30 μL of LaGM (30 mg mL^{-1}) was added to the supernatant, followed by vortexing for 2 min, shaking for 10 min at room temperature, centrifugation for 5 min at 10000 rpm, and magnetic separation. After removing the supernatant, the precipitate was washed three times with water, the peptides were eluted from precipitate with eluent of acetonitrile (80%) and trifluoroacetic acid (1%) twice and then lyophilized.

2.3 Identification of mass spectra

The lyophilized peptide samples redissolved in 98% water containing 2% acetonitrile and 0.1% formic acid. The online Nano-RPLC was performed on liquid chromatography Eksigent nanoLC-Ultra™ 2D system. The dissolved sample was pre-loaded onto the C_{18} column (30 mm × 100 μm, 3 μm, 150 Å) at a flow rate of 2 μL min^{-1}, followed by desalination for 10 min. Analytical column was C_{18} reverse phase column (150 mm × 75 μm, 3 μm, 120 Å), and the gradient program for the chromatographic separation was as follows: 0–42 min, 5%–25% B; 42–56 min, 25%–40% B; 56–64 min, 80% B; 64–70 min, 5% B. The mass spectrometer parameters of TripleTOF 5600 system (AB SCIEX) binding nanospray III ion source (AB SCIEX) were optimized as follows: 2.4 kV of spray voltage, 30 psi of curtain air pressure, 5 psi of atomization gas pressure, 150 °C of heating temperature, and 250 ms of scan time of single TOF-MS altas. Every IDA cycles collected up to 35 secondary patterns whose charge among 2^+–8^+ with the count was greater than 100 per second, and the cumulative time for each secondary pattern was 80 ms. Besides, the time of each cycle was fixed at 2.5 s, the collision cell energy was set to be suitable for all of the precursor ion collision-induced dissociation (CID), and the dynamic exclusion was set to 11 s.

2.4 Data analysis

The original wiff filed that mass spectra were collected using Protein Pilot Software v.4.5 (AB SCIEX, USA) software for data analysis and retrieval processing, database was Homo sapiens-specific database of Uniprot library (containing 20210 protein sequences, download on January 2, 2015), the search parameters were non digestion, phosphorylation and biological modification, the retrieval mode was thorough analysis, and the false positive rate was controlled to 1% FDR.

2.5 Bioinformatics analysis

Protein interaction network was analyzed on String10 (http://www.string-db.org/). String database search system was used to search a known interaction between the protein and the predict protein, which included both direct interaction and indirect physical interaction between proteins. Protein clustering was analyzed on PANTHER (http://www.pantherdb.org/). Panther was based on Gene Ontology database of protein classification.

3 Results and discussion

3.1 Urine peptidomic and analysis at protein level