Journal of Chromatography A, xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

The use of chemical probes to detect the proteomics of renal tubular injury induced by maleic acid

Hugo You-Hsien Lin[a,b,c,d,e], Chan-Jung Liang[f], Meng-Chieh Liu[g], Mei-Fang Huang[g], Jung-San Chang[i,j], Shih-Shin Liang[g,h,*]

[a] Internal Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[b] Division of Nephrology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[c] Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[d] Department of Physiology & Biophysics, University of California at Irvine, Irvine, CA 92697, USA
[e] UC Irvine Diabetes Center, University of California at Irvine, Irvine, CA 92697, USA
[f] Department of Medical Science Industry, College of Health Sciences, Chang Jung Christian University, Taiwan
[g] Department of Biotechnology, College of Life Science, Kaohsiung Medical University, Kaohsiung, Taiwan
[h] Institute of Biomedical Science, National Sun Yat-Sen University, Kaohsiung, Taiwan
[i] Division of Gastroenterology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, 100Shih-Chuan 1st Road, Kaohsiung 80708, Taiwan
[j] Department of Renal Care, College of Medicine, Kaohsiung Medical University, Taiwan

ARTICLE INFO

Article history:
Received 28 November 2017
Received in revised form 29 May 2018
Accepted 17 June 2018
Available online xxx

Keywords:
Maleic acid (MA)
Chemical probes
Human kidney cell lines (HK-2)
Protein–protein interactions
Proteomics

ABSTRACT

Maleic acid (MA), an industrial raw material, was found to be illegally added to edible starch-based food products in Taiwan in 2013, a practice unheard of in most of the world. MA has been associated with renal dysfunction in many experimental animal studies. In this study, we developed chemical probes to investigate protein–protein interactions between MA and renal proteins. In the fabrication of the MA probes, we used silicon dioxide (SiO_2) modified with a silanized linker (3-aminopropyl triethoxylane, APTES) to generate MA with APTES–SiO_2 particles. The probes were then incubated with the cell lysates of normal human kidney cell lines (HK-2) and subjected to MS/MS for identifying several MA-related proteins, including nucleophosmin, neutral alpha-glucosidase AB, translocon-associated protein subunit alpha, elongation factor 1-gamma, 60S acidic ribosomal protein P0-like, and heat shock protein (HSP 90-alpha and beta). Based on our findings, we believed that the probe can potentially be used to identify and detect the target proteins and help characterize a network of MA protein–protein interactions.

1. Introduction

Maleic acid (MA) is frequently used as a surfactant or stabilizer in the manufacture of various industrial products [1]. In most of the world, while MA might be used in the manufacture of food containers, it is not used in the production of food. In Taiwan, however, there have been instances in which some food manufacturers have unscrupulously added MA as an emulsifier to their starch based food products, including frozen dumplings and glutinous desserts [2]. This is alarming because MA has been found in animal models to cause various renal dysfunctions, including type II renal tubular acidosis (RTA II) [3–5], Fanconi's syndrome (FS) [6–9], renal glycosuria, phosphaturia and aminoaciduria [10–12]. MA has also been found to induce renal proteinuria [13] as well as Na/K pumping inhibition and Na(+)/K(+)-ATPase activity [5,8,14]. It also stimulate glutamine metabolism in renal cortical tubules of dogs and rats [15–17], and reduces concentrations of CoA-SH and acid-soluble acyl-CoA in the mitochondria in rat kidney [18].

Gel-free shotgun proteomics coupled with liquid chromatography tandem mass spectrometry (MS/MS) [19] and multidimensional gel separations with liquid chromatography tandem MS have been used to measure the expression of specific proteins and establish the pathways of several diseases [20–22]. However, although proteomics can be used to identify many candidate proteins, various complementary methods such as mass spectrometry are needed to isolate the proteins that can serve as biomarkers in the screening and verification of specific diseases such as can-

* Corresponding author at: Department of Biotechnology, College of Life Science, Kaohsiung Medical University, 100, Shih-Chuan 1st Road, Kaohsiung, 80708, Taiwan.
E-mail addresses: yukenlin@yahoo.com.tw (H.Y.-H. Lin), d9101308@hotmail.com (C.-J. Liang), liumengchieh@icloud.com (M.-C. Liu), grace030604@yahoo.com.tw (M.-F. Huang), cjs@kmu.edu.tw (J.-S. Chang), liang0615@kmu.edu.tw (S.-S. Liang).

https://doi.org/10.1016/j.chroma.2018.06.040
0021-9673/

cer [21–25]. Other confirmations and validation methods include western blotting, qPCR [23], microarray chips [24,25], and triple quadrupole MS using the multiple reaction monitoring (MRM) [26–28]. Nonetheless, given the sheer number of candidate proteins, we are still in need of more disease-specific proteomics procedures coupled with an MS instrument to better narrow down the number of possible candidate proteins.

For this research, we created chemical probes to detect MA-associated proteins utilizing methods developed in previous experiments [29–31]. We used *N*-hydroxysuccinimide/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (NHS/EDC) to modify MA on a chemical probes silicon dioxide and check this MA probe with fluorescence microscopy and infrared spectroscopy. In this study, we incubated normal human kidney cell lines (HK-2) with MA chemical probes to create a model of human MA-related nephropathy. We then sought to identify specific binding proteins using MS/MS and Mascot searching engine (SwissProt database). These MA-associated proteins were then correlated with the STITCH database for better specificity.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals and reagents, including hydrochloric acid, ammonium hydrogen carbonate (NH_4HCO_3), ethanol, urea, sodium hydroxide, sodium dodecyl sulfate (SDS), and acetonitrile (MeCN) were obtained from J. T. Baker (Phillipsburg, NJ, USA). Trifluoroacetic acid (TFA), DL-dithiothreitol (DTT), EDC, sodium acetate, and sodium cyanoborohydride ($NaBH_3CN$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trypsin was purchased from Promega (Madison, WI, USA). Sodium dihydrogen phosphate (NaH_2PO_4), potassium dihydrogen phosphate (KH_2PO_4), formaldehyde solution (36.5%–38% in H_2O), iodoacetamide (IAM), formic acid (FA), potassium chloride (KCl), and sodium chloride (NaCl) were obtained from Sigma (St. Louis, MO, USA). MA was purchased from Chem Service (West Chester, PA, USA). Formaldehyde-D_2 (20% solution in D_2O) was obtained from Isotec Corp. (Miamisburg, OH, USA). APTES, potassium bromide (FTIR grade), and NHS were purchased from Alfa Aesar (Heysham, LA3 2XY, UK). Deionized water was produced in a Millipore water system with a resistivity of 18.2 MΩ.

2.2. Synthesis of MA probes

A total of 150 mg of silicon dioxide (SiO_2, 400 mesh, approximately 40 μm; Acros organics, Geel, Belgium) was activated by vortex with 0.5 M NaOH and 0.5 M HCl, and then washed with ethanol and distilled water to remove residual HCl. NaOH. SiO_2 was silanized with APTES (5% in ethanol) over 12 h. The SiO_2 powder was washed twice with 10 mL of ethanol and then stored in an oven overnight at 50 °C. Next, 15 mg of MA was activated using 13 mg of EDC with 5 mg of NHS in 5 mL of deionized water. The activated MA solution was interacted with APTES-modified SiO_2 in an eppendorf tube overnight. The modified SiO_2 particles were then washed with deionized water and ethanol and baked in an oven (50 °C).

2.3. Characterization of MA probes

MA-APTES-SiO_2, MA, and APTES-modified SiO_2 were individually mixed and ground with KBr (FTIR grade) to generate flat wafers by oil hydraulic press. The flat wafers were analyzed by IR spectroscopy using a Perkin-Elmer Spectrum RX1 spectrometer (Canton, MA, USA).

APTES-modified SiO_2 and bare SiO_2 were mixed with 5 mg of BDP FL NHS ester (Hallandale Beach, FL, USA) and 500 μL of PBS. Both samples were then placed on a microscope slide with its four edges covered with plate mats. The samples were then anatomized using Fluorescence Microscopy (Leica DMI6000, Leica Microsystems, Mannheim, Germany).

2.4. HK-2 cells culture and protein concentration

HK-2 cells (American Type Culture Collection (ATCC), CRL-2190, Manassas, VA, USA, CRL-2190) were cultured in a Keratinocyte serum-free medium (available from GIBCO BRL #10724-011) with 5 ng/mL recombinant epidermal growth factor and 40 ug/mL bovine pituitary extract. The cells were cultured in a 100 mm dish in a 5% CO_2 incubator at 37 °C until they reached 80% confluence. The HK-2 cells were lysated by RIPA buffer (pH 7.5, 50 mM Tris-HCl, 0.1% SDS, 150 mM NaCl, and 1% NP-40) to which one tablet (10 mL) of Roche mini complete protease inhibitor was added.

2.5. Conditions of MA probes incubated with HK-2 cells lysate

Ten mg of each APTES-modified probe (Probe 1) and MA probe (Probe 2) were placed into different eppendorf tubes each with 100 μg (Bradford assay determined protein concentration) of HK-2 cell lysates, respectively. 300 μL of phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 8 mM NaH_2PH_4, and 1.4 mM KH_2PO_4) was used to dilute HK-2 cell lysates. Probes 1 and 2 were both incubated at 37 °C for 4 h. The supernatant was then removed by centrifugation. MA probes with bound proteins were washed three times with 200 μL of PBS buffer.

2.6. Tryptic digestion and dimethyl labeling

Urea (48 mg) and 0.1 M DTT (5 μL) were added to eppendorf tubes containing individual chemical probes placed in 100 μL of 50 mM NH_4HCO_3, and incubated for 1 h at 37 °C. Then, 5 μL of 50 mM IAM was added for alkylation at 4 °C for 2 h. After reduction and alkylation, individual samples were diluted with 400 μL of 50 mM NH_4HCO_3 (less than 2 M urea) and 0.2 μg of trypsin was added for digestion. After 4 h, another 0.2 μg of trypsin was added and incubation was continued at 37 °C for another 18 h. Finally, tryptic peptides were removed from the MA probes with 50% and 95% MeCN and dried for 3 h in a vacuum centrifuge. Next, the lyophilized peptides were redissolved in 180 μL of 100 mM sodium acetate. Probe 1 and 2 peptides were labeled using 10 μL of 4% formaldehyde-H_2 and 10 μL of 4% formaldehyde-D_2, respectively, and each vortexed for five min. Ten μL of 0.6 M reduced reagent sodium cyanoborohydride was added to each sample for 1 h. The Probe 1 and Probe 2 samples were acidized with 10% TFA for desalting. The labeled solutions, three for each probe, had pHs ranging between 2 and 3. Each Probe 1 sample was paired with a Probe 2 sample and combined using a reverse-phase homemade C18 cartridge desalting kit. After being dried in a vacuum for 3 h, the labeled peptides were identified and characterized using nano-LC–MS/MS.

2.7. Instrumentation: nano-LC–MS/MS and Mascot search

Reverse-phase nano-LC separation was performed using a Waters ACQUITY nano flow system (nano UPLC, Waters Corp., Manchester, UK). For this, a trapped column (20 mm × 180 μm) and Waters BEH C18 column (i.d. 75 μm × 150 mm, 1.7 μm particle size) were used. The C18 column was loaded with 3 μL of labeled sample from an eluted fraction. UHPLC flow rates were 5 μL/min (loading pump) and 300 nL/min (gradient pump) with two mobile-phases buffer A consisted of 0.1% FA in water and buffer B consisted of 0.1% FA in 100% MeCN. The linear gradient is set in the following sequence: initial 5% (B) for 2 min, 5%–40% (B) for 40 min, 40%–95% (B) for 8 min, and held at 95% (B) for 2 min.

Please cite this article in press as: H.Y.-H. Lin, et al., The use of chemical probes to detect the proteomics of renal tubular injury induced by maleic acid, J. Chromatogr. A (2018), https://doi.org/10.1016/j.chroma.2018.06.040