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Original article

Label-free quantification of peptides in solution by disposable patterned hydrophilic chip based MALDI imaging

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ABSTRACT

Quantification of a mixture of peptides in solution was achieved by disposable patterned hydrophilic chip based matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI MSI). Compared with other quantitative methods for peptides in solution, this method is label-free and does not require separation of the multiple components of the solution before analysis. Uniform hydrophilic spots and high mass accuracy measurements provided confident identification and quantitative analysis of imaged compounds. The linear correlation between concentration and grayscale of image in the range of 5 fmol/μL to 1 pmol/μL was obtained for all four peptides. Good sensitivity and excellent reproducibility were also achieved. The method expands the application of MALDI MSI from tissues to solutions.

1. Introduction

Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI MSI) has become a powerful tool in biological, chemical and clinical applications since its introduction in 1980s due to its spatial resolution and label-free detection properties [1]. With MALDI MSI, both chemical information and spatial distribution of each analyte can be obtained through direct analysis of thin tissue sections [2]. Compared with other imaging methods, MALDI MSI can determine the distribution of multiple compounds in a single measurement and may provide a higher mass range and mass resolution [3]. Nowadays, MALDI MSI has been used to determine the local concentrations of proteins, peptides, lipids, drugs, metabolites, and other compounds in biological tissues [4–11] and polymers [12].

To the best of our knowledge, MALDI MSI has been applied mostly to determine the localized distribution of analytes in solid matrices (such as tissues and polymers) and was successfully employed in the determination of some biomarkers in tumor tissue sections which can provide not only spatial distribution, but also semi-quantitative information in tissues [13–15]. However, direct quantitative analysis of interested peptides and proteins in physiological fluids (serum, urine) is seldom realized by this kind of imaging technique. The stable-isotope labeling (SIL) technique, including chemical, enzymatic and metabolic methodologies, is a powerful method for absolute and relative quantitation [16,17]. However, these techniques necessitate expensive labeling reagents (*e.g.* ICAT, SILAC, iTRAQ) and complicated, time-consuming separation steps [18]. The novel label-free method based on liquid chromatography requires complicated computation and reliable separation as the outcome is strongly dependent on the liquid chromatography (HPLC) conditions [19]. There are several key advantages of MALDI-MSI over traditional quantitative methods, namely, no requirement to label the analyte due to its identification by its mass and fragmentation pattern and the ability to detect multiple analytes from one experiment (with full mass range capabilities).

A patterned hydrophilic chip based MALDI MSI is reported here to accomplish quantitative analysis of peptides in solution *via* image analysis. The patterned hydrophilic chip is a hydrophilic-hydrophobic hybrid chip which contains two different areas with the surface of each modified by different materials to achieve hydrophilic or hydrophobic properties [20,21]. The use of the patterned hydrophilic chip gives a spatial delimitation of samples and insures the reproducibility of sample distribution. The images from MALDI MSI can reflect the distribution of analytes in hydrophilic spots and then quantitation information in solution [22].

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The aim of this study was to broaden the application of MALDI MSI from solid to solution samples and develop a direct quantitative analysis of peptides in solution. The label-free and separation-free utilization properties of the patterned hydrophilic chip based MALDI MSI makes it a simple, feasible and potential quantification method.

2. Experimental

Indium-Tin Oxide (ITO) glass was purchased from Hudson Surface Technology (Old Tappan, NJ). Polydimethylsiloxane (PDMS, Sylgard184) was obtained from Dow Corning (Midland, MI). Four peptides (Bradykinin fragment 1–7, Angiotensin II (human), P14R (synthetic peptide), and ACTH fragment 18–39), the peptide standards for MALDI MS, and α-cyano-4-hydroxycinnamic acid (99%, CHCA) were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (HPLC grade) and trifluoroacetic acid (99.5%) were purchased from J&K Chemical (Beijing, China). All aqueous solutions were prepared using deionized water (18.2 MΩ cm resistivity) purified with an A10 Milli-Q water purification system (Merck Millipore, Billerica, MA).

In order to obtain a hydrophilic-hydrophobic hybrid chip, screen-printing method was applied here using ITO as hydrophilic material and PDMS as hydrophobic material. The screen-printing model was designed by Computer Aided Design (CAD), then PDMS was screen-printed on ITO glass as a hydrophobic surround [23]. A TORCH T3244 manual screen-printing machine (Beijing Torch Co., Ltd., China) was used to fabricate the patterned hydrophilic chip. The mesh number of the white area was 200 to make the hydrophobic polymer pass through.

Samples of 1.5 μL containing the same concentrations of four peptides were applied into hydrophilic spots on the chip by the dried-droplet method and allowed to dry under ambient conditions before spectral acquisition. The matrix used was 10 mg/mL CHCA in 50% acetonitrile and 0.1% trifluoroacetic acid.

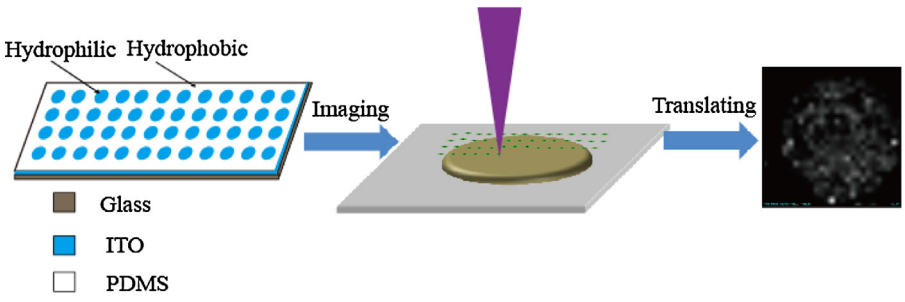
MALDI MS imaging was carried out on an AB Sciex 4800 plus MALDI–TOF/TOF MS equipped with a Nd:YAG laser (emitting at 355 nm, operated at 200 Hz). The images were recorded in reflectron positive ion mode and externally calibrated with "TOF/TOF calibration" standard solution (AB Sciex, Framingham, MA). Both 4000 series Explorer (AB Sciex, Framingham, MA) and 4000 imaging softwares were used for data acquisition and processing. The laser energy and the raster step size were set at 18 μJ and 100 μm, respectively. Each mass spectrum per pixel was collected by averaging the signals of 200 individual laser shots at fixed position.

Biomap software (Novartis, Basel, Switzerland) was used for image analysis. The obtained spectral data of each scan were transformed to image data using Biomap software in which the mass range, bin size and layout were fixed for all the samples. Then a desired mass was extracted in the spectral profile to plot the image of this species. After transformation, the data were saved as 8-bit grayscale images and analyzed using MATLAB software (version 4.0, The MathWorks, Inc., Natick, MA) for the sum of grayscale of region of interest (ROI) in each image [24]. ROIs designed in MATLAB analysis were the same rectangles including the hydrophilic spots in the chip. For 8-bit images, the pixel values ranged from 0 to 255. The external standard method was used to quantify peptides in solution. The relationship between the sum of grayscale for each extracted image and the sample concentration was plotted with best-fit regression analysis.

3. Results and discussion

The patterned hydrophilic chip (Scheme 1) used in this study was a hybrid hydrophilic-hydrophobic glass plate containing a collection of hydrophilic ITO spots surrounded by hydrophobic polymer material. To obtain more reliable results, the sampling areas were restricted using the hydrophilic spots in the chip which eliminated heterogeneity of sample preparation, and therefore the ionization effect. The patterned hydrophilic chip based MALDI MSI quantification analysis procedure was illustrated in Scheme 1. Considering the shape of the droplet, the hydrophilic spots were designed to be circles. Then the hydrophilic-hydrophobic hybrid chip was prepared as described in Experimental section, fixed on a MALDI plate and submitted to MALDI TOF MS. Both absolute and relative quantitative analyses can be achieved using the patterned hydrophilic chip based MALDI MSI quantification analysis.

A patterned hydrophilic chip can be prepared by many different methods, such as screen-printing, spin-coating, and ink-jet printing. The hydrophilic or hydrophobic polymer materials were deposited onto the substrate to form desired structures. Screen-printing was simpler and cheaper than other methods and selected in this work. In screen-printing, a design was imposed on a fine mesh screen, with blank areas coated with an impermeable substance. Since the MALDI ionization process is enhanced by the presence of a conductive surface (ITO coated glass slides, stainless steel plates, gold-plated stainless steel plates, *etc.*) under the sample and the surface of ITO glass is hydrophilic due to metallic oxide and hydroxyl and carbonyl group content, ITO glass was selected as the hydrophilic surface of our hybrid chip. The screen-printing model contained an array of impermeable spots that would result in exposed ITO hydrophilic spots on the chip. PDMS was a hydrophobic polymer that was commonly used for making microfluidic chips and in the present study, it was chosen as the hydrophobic material for the areas surrounding the spots. The PDMS prepolymer and curing agent (Sylgard 184, Dow Corning) were mixed in a 10:1 ratio (w/w). Then, air bubbles were removed by applying a vacuum. After screen-printing PDMS on ITO glass, the patterned hydrophilic chip was cured at 60 °C for 2 h. Fig. S1 in Supporting information shows a comparison of wetting states and



Scheme 1. Schematic illustration of the patterned hydrophilic chip based MALDI MS imaging procedure.