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Matrix Pre-coated Targets for High Throughput MALDI Imaging of Proteins

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Abstract

We have developed matrix pre-coated targets for imaging proteins in thin tissue sections by MALDI MS (matrix-assisted laser desorption/ionization mass spectrometry). Gold covered microscope slides were coated with sinapinic acid (SA) in batches in advance and were shown to be stable for over 6 months when kept in the dark. The sample preparation protocol using these SA pre-coated targets involves treatment with diisopropylethylamine (DIEA)-H₂O vapor, transforming the matrix layer to a viscous ionic liquid. This SA-DIEA ionic liquid layer extracts proteins and other analytes from tissue sections that are thaw mounted to this target. DIEA is removed by immersion of the target into diluted acetic acid (AcOH), allowing SA to co-crystallizes with extracted analytes directly on the target. Ion images (3–70 kDa) of sections of mouse brain and rat kidney at spatial resolution down to 10 μ m were obtained. Use of pre-coated slides greatly reduces sample preparation time for MALDI imaging while providing high throughput, low cost, and high spatial resolution images.

Introduction

MALDI IMS (matrix-assisted laser desorption/ionization mass spectrometry) is a highly effective discovery tool because it provides spatial information on a variety of compounds simultaneously without the need for *a priori* knowledge of these compounds. Such molecular mapping can be accomplished at 5–10 μ m spatial resolution from thin tissue sections of nearly any size with unmatched molecular specificity. Although careful sample preparation is important in achieving high quality images, it can be time consuming and generally requires special equipment for matrix deposition, *e.g.*, nebulizer,^{1–2} modified inkjet printer,³ robotic droplet depositor,⁴ chemical printer,⁵ spray depositor⁶ and vibrational vaporizer⁷. In addition although high molecular weight species (30 to over 200 kDa) have been measured directly from tissue sections under special conditions^{8–11}, imaging high molecular weight species (>50 kDa) remains challenging. In general, 30 minutes or more is required to prepare a tissue section for imaging, making high throughput,

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robust and cost effective matrix deposition method highly desirable. Matrix pre-coated MALDI targets have been reported for continuous sample deposition^{12–13}, lipid imaging using 2,5-dihydroxybenzoic acid (DHB)¹⁴, derivatization reagents¹⁵ and application of 1,5-diaminonaphthalene(DAN)¹⁶. These matrix pre-coated targets are suitable for imaging lipids, small peptides and pharmaceuticals but have not proven effective for imaging proteins.

We report here a sample preparation method employing targets homogeneously pre-coated with sinapinic acid (SA) for imaging proteins (~3–70 kDa) with high spatial resolution (down to 10 μ m) and high throughput because there is no matrix deposition step at the time of analysis, only a fast hydration and rinsing step. Briefly, SA on a pre-coated slide is converted to an ionic liquid through exposure to an organic diisopropylethylamine (DIEA) vapor and thin tissue sections are placed on top. A single step of immersing the target into diluted acetic acid (1%) for 60 seconds completes the sample preparation (Figure 1). Matrix pre-coated slides were prepared by casting matrix solution on clean gold coated microscope slides. The sample preparation protocol was evaluated for the thickness of matrix coating, the thickness of tissue section, reproducibility of imaging of serial sections and shelf life of the prepared slides. Ion images are presented for mouse brain, rat kidney and rat brain sections.

Experimental and materials

Ethanol, methanol, acetonitrile (ACN), and acetic acid were purchased from Fisher Scientific (Suwanee, GA), and xylene from Acros (Morris Plains, NJ), Dimethyl sulfoxide (DMSO), isopropanol, diisopropylethylamine (DIEA), potassium hydroxide and hydrogen peroxide (30%) from Sigma-Aldrich (Milwaukee, WI). Sinapinic acid was purchased from Oakwood Products, Inc (SC) and recrystallized twice with 70% ACN. Water-white glass microscope slides with a Titanium adhesion layer and 50 nm thick Au coating were purchased from Deposition Research Lab. Inc. (St. Charles, MO). Frozen rat brain, mouse brain, chicken liver and rat kidney were obtained from Pel Freez (Rogers, AR), and stored at -80°C .

Preparation and use of matrix pre-coated slides on gold coated slides

Each gold coated microscope slide (7.5×2.5 cm) are cut by a diamond glass cutter to the size desired by the user. We typically cut each slide to give 5 targets of 2.5×1.5 cm. The targets were cleaned by immersing them into a solution of hydrogen peroxide (30%) and saturated potassium hydroxide in isopropanol at 2:1 ratio for 3 minutes, rinsed with MilliQ water (from a Milli-Q Advantage A10 Ultrapure Water Purification System, Millipore, Billerica, MA) and absolute alcohol, and air dried.

SA coating of the targets were done by immersing them into a 3 mL of SA solution (100 mg SA in 10 mL H_2O , 5 mL ethanol, 1 mL acetic acid, and 650 μL chloroform) in a Petri dish (35×10 mm). The solution was left for evaporation for about 2 hours in the hood. The targets were then rinsed with MilliQ water and dried under ambient conditions. The weight of the gold coated slides was measured before and after the SA coating, the difference of the weight was divided by the area of the slides ($2.5 \text{ cm} \times 1.5 \text{ cm} = 3.75 \text{ cm}^2$) to give the weight/

area of each slide. Generally, this procedure provides a coating of SA of approximately 2.6–2.7 mg/cm². The targets were carefully placed on a piece of emery paper (8000 grit, Scientific Instrument Services, Ringoes, NJ) with the matrix side down and gently polished to remove larger crystals. The pre-coated targets were preserved under dark conditions at room temperature, and when stored in this way, they gave equivalent performance when stored for over 6 months.

Each SA pre-coated target is placed in a 35 × 10 mm Petri dish and this dish was placed in a 60 mL glass jar (Fisher, 02-911-763) containing 200 µL of DIEA and 900 µL of H₂O under room temperature. The jar was then sealed for 7 minutes. The target was taken out and placed in the cryostat chamber to cool to temperature of –20°C. Thin sections of different tissues were placed on the top of the matrix coating and the slide was immediately immersed into 20 mL of 0°C cold 1% AcOH for 60 s. The solution was agitated for 10 seconds so that the tissue on the surface was removed. The slide was rinsed again in a second bath of 1% AcOH and dried under ambient conditions for subsequent imaging.

Serial tissue sections were subjected to the standard hematoxylin and eosin (H&E) staining¹⁷. Optical images of the H&E stained section were acquired with a Mirax slide scanner from Zeiss at 0.23 µm resolution.

MALDI MS analyses were performed on a Bruker Autoflex Speed mass spectrometer in positive ion linear mode using FlexControl 3.3 software. Approximately 100 to 200 laser shots/spot were acquired with a 1kHz repetition rate Smartbeam II Nd:YAG laser (355 nm). Image production and analysis was performed using FlexImaging 3.0 and FlexAnalysis 3.3.

Results and discussion

Activation of the stored SA pre-coated slide was performed by treatment with an organic amine (DIEA) and water vapor, converting SA on the surface to an ionic liquid (SA-DIEA-H₂O). After tissue placement on the activated slide, the liquid matrix extracts proteins from tissue section. The SA-DIEA-H₂O layer is viscous, minimizing delocalization of compounds after tissue placement. Results presented in the following examples show no significant delocalization at the spatial resolutions tested. When the slide is immersed into diluted AcOH, DIEA is removed from the liquid film and the SA precipitates and co-crystallizes with the extracted proteins.

Assessing tissue thickness and the amount of matrix coating

Tissue sections of the same thickness (liver sections 3 µm thick, Figure 2A) were placed on the matrix pre-coated slide with different amounts of matrix coating (weight of matrix/cm²). It was observed that the peak intensity in the average mass spectrum is maximized when the thickness of the coating is 1.1 mg/cm² or more.

In our protocol, the tissue section was removed at the last step and therefore the thickness of tissue section has little impact on the mass spectral profile (Figure 2B). This thickness independence is different from the pre-coated target for imaging lipids¹⁶, where the tissue section is preserved on the surface and the thinner the section, the better quality of the mass

spectrum. Indeed, in the current case where the same protocol was used except that the tissue sections were kept on the SA slide, we observed a reduced sensitivity (S-Figure 1).

A rat kidney section of 12 μm thickness was imaged at 120 μm spatial resolution producing high quality ion images in the m/z range between 3000–70,000 (average spectrum shown in Figure 3). All the ion images matched well with the optical images of H&E stained serial sections (Figure 4). Similar results were obtained for mouse brain images when imaged in the 3–70 kDa range at both low spatial resolution (100 μm , shown in S-Figure 2 and 3 and at high spatial resolution (10 μm , shown in Figure 5).

Matrix pre-coated slides can be made beforehand in batches, enabling quality control assessment of each slide either optically or gravimetrically. Using targets with carefully controlled coating thickness, matrix crystal size distribution, and surface homogeneity, high reproducibility can be achieved, as demonstrated in Figure 6, where 4 serial rat brain sections were placed onto SA pre-coated slides that had been stored for 1, 2, 30 and 180 days. Nearly identical images were obtained demonstrating that SA pre-coated slides can provide reproducible performance and have great stability over at least several months.

We have also investigated the effectiveness of different target materials for pre-coated slides, including: glass, stainless steel, copper, aluminum, silicon and gold, following application of SA solution. A gold surface gave the best performance using a homogenous SA coating. Although homogenous SA coatings can be obtained on almost any surface using the TM sprayer, we have determined that gold surfaced slides with an SA coating had sufficient stability to sustain the transformation to ionic form and survive the immersion into the 1% AcOH. On non-gold surfaces, at the final step of immersing into the AcOH, the SA coating sometimes peeled off the surface resulting the failed sample preparation.

For SA pre-coated slides, under the condition where SA was not transformed to SA-DIEA- H_2O , or just transformed to SA-DIEA without water in the layer, the mass spectra produced were of significantly poorer quality.

Conclusions

The SA pre-coated slides can be prepared in advance and preserved under dark conditions for a long period of time, a significant convenience for the end user. These targets can be used to image proteins at high spatial resolution (down to 10 μm) and image high molecular weight species up to about 70 kDa. Since there is no need for expensive matrix coating robotics for matrix deposition, use of pre-coated slides are cost effective and do not require special expertise on the part of the user.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Sample preparation with a sinapinic acid pre-coated slide (Yellow: SA; green: SA-DIEA-H₂O)

Figure 2.
(A) 3 μm thick chicken liver sections placed on slides with different amounts of SA coating;
(B) different thicknesses of rat brain sections imprinted on SA pre-coated slides with a coating of 2.7 mg/cm². Average spectra were taken from 100 locations on the tissue.

Figure 3.
Average spectra from a rat kidney from the images in Figure 4. (A) 3–30 kDa; (B) 30–70 kDa. These were acquired from two serial sections.

Figure 4. Representative m/z ion images from 3–30 and 30–70 kDa from two serial sections of a rat kidney (scale bar: 2 mm). M/z values are noted below the images.

Figure 5.

Ion images at 10 μm spatial resolution from a mouse brain section. M/z values are noted below the images. Scale bars: 200 μm

Figure 6.

Reproducibility and stability of SA pre-coated targets. Four serial sections of a rat brain were imaged at 150 μm spatial resolution using SA pre-coated slides stored for 1, 2, 30 and 180 days. M/z values are noted above each column.