Published in final edited form as:

Anal Chem. 2013 March 5; 85(5): 2907–2912. doi:10.1021/ac303554e.

Matrix Pre-Coated Targets for Direct Lipid Analysis and Imaging of Tissue

Junhai Yang¹ and Richard M. Caprioli^{*,1,2}

¹Department of Biochemistry, Vanderbilt University, Nashville, Tennessee, USA

²Departments of Chemistry, Pharmacology and Medicine, and the Mass Spectrometry Research Center, Vanderbilt University, Nashville, Tennessee, USA

Abstract

We have developed targets pre-coated with matrix for imaging lipids in tissues using MALDI-MS. Thin tissue sections (rat kidney and mouse or rat brains) were placed onto 2,5-diaminonaphthalene pre-coated targets (prepared beforehand by a protocol utilizing sublimation) and were washed with ammonium formate solution. After a brief drying period, the target slides were imaged by MALDI MS. The resulting images from these sections were of equivalent quality to those obtained using the usual post-coating approach such as sublimation and spraying in term of the sharpness of substructures in the images demonstrated by imaging at spatial resolutions of 100, 10 and 5 μm . Matrix pre-coated targets have a shelf-life more than 6 months when kept in a dark, non-humid environment such as a non-transparent desiccator.

Introduction

MALDI Imaging Mass Spectrometry (MALDI IMS)¹ has seen wide use in the mapping of peptides, proteins, lipids, metabolites and pharmaceuticals in thin tissue sections.²⁻⁵ As in most if not all analytical measurements, sample preparation is a key step in obtaining high quality data. For imaging, this involves tissue collection, sectioning, chemical pre-treatment and matrix application. The quality of the coated matrix layer (the size of matrix crystals and the homogeneity and stability of the coating) directly impacts the quality of the ion images produced. Currently, typical matrix application widely used in the field employs manual spray, robotic spray and spotting, inkjet printing, and sublimation.⁶ However, these methods either show less than acceptable reproducibility, are time consuming at the point of use, or require expensive robotic equipment.

We have developed an approach to matrix deposition for MALDI Imaging MS that circumvents these problems for lipid analysis through the use of matrix pre-coated targets. This effectively allows the user to make high quality targets in advance that are stable on storage and robust while still delivering high quality performance. As a result, when an experiment is begun, only a few minutes and a minimum amount effort is needed for the user to produce an image ready target.

The concept of “matrix pre-coated target” has been used previously for non-imaging purposes.⁷⁻¹⁰ We have developed pre-coated matrix targets for MALDI IMS, using a 2,5-

*CORRESPONDING AUTHOR. Mass Spectrometry Research Center, Vanderbilt University, 465 21st Avenue South, 9160 MRB 3, Nashville, TN 37232-8575. Phone (615) 343-2700, Fax (615) 343-8372. .

Supporting Information The ion images from CHCA and DHN pre-coated targets are detailed in the Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

dihydroxybenzoic acid (DHB) for imaging lipids and other small molecules,¹¹ such as the anti-tuberculosis drug isoniazid with trans-cinnamaldehyde pre-coated targets.¹² However, with a DHB pre-coated target, the hydrophilic nature of DHB and its solubility in water was found in practice to cause delocalization of analytes and preclude signal enhancement procedures such as washing of tissue sections after placement on the target due to loss of the matrix.

In this paper, we describe the preparation and performance of matrix pre-coated targets and the evaluation of performance parameters such as the effect of the thickness of the matrix coating and tissue section. In this work, we have used 1,5-diaminonaphthalene (DAN), 2,6-dihydroxynaphthalene (DHN) and alpha-cyano-4-hydroxycinnamic acid (CHCA) for pre-coated matrix application. The result of these studies is a simple and highly sensitive method for imaging lipids in tissue with a spatial resolution up to 5 μm .

Experimental and materials

Ethanol, methanol, acetonitrile (ACN), and acetic acid were obtained from Fisher Scientific (Suwanee, GA), and ammonium formate, alpha-cyano-4-hydroxycinnamic acid (CHCA), 1,5-Diaminonaphthalene (DAN) and 2,6-dihydroxynaphthalene (DHN) from Sigma-Aldrich (Milwaukee, WI). Conductive indium tin oxide (ITO) coated microscope glass slides were purchased from Delta Technologies (Stillwater, MN). Teflon coated microscope slides were purchased from Electron Microscopy Sciences (PA, 63415-08). Frozen rat brain, mouse brain, and rat kidney were obtained from Pel Freez (Rogers, AR), and stored at -80°C .

A simple sublimation apparatus was constructed from a condenser and flask (from Chemglass Life Sciences, Vineland, NJ)¹³. A microscope slide of 7.5×2.5 cm was taped to the bottom of the condenser. The apparatus was coupled to a rough pump and a digital thermocouple vacuum gauge controller and was placed on a sand bath heated by a hot plate (VWR 47751-150). The temperature was monitored with a digital thermometer.

Matrices were recrystallized according to common organic chemistry methods¹⁴ by using 70% ACN for CHCA, or acetone for DAN. DHN was used without recrystallization.

Preparation of matrix pre-coated slides by sublimation

ITO coated microscope glass slides (2.5×7.5 cm) were cleaned by immersion in a saturated sodium carbonate solution for 4 hours, rinsed with deionized water (2 minutes) and MilliQ water (from a Milli-Q Advantage A10 Ultrapure Water Purification System, Millipore, Billerica, MA) (2 minutes), and placed in an 85°C oven for 1 hour to dry. A stainless steel plate was attached to the bottom of the condenser of the sublimation apparatus, and the ITO glass slides were attached to this plate with copper tape (Electron Microscopy Sciences, 77801). Approximately 100 mg of one of the matrices (DAN, DHN or CHCA) was placed in the flask of the sublimation apparatus along with a magnetic stirring bar. Twenty mL of acetone was added to the flask that was then placed on the sand bath at a temperature set for each matrix, stirred under a gentle stream of air. A homogenous thin matrix film formed after the acetone was evaporated. The condenser and the flask were assembled and sublimation was carried out at 25 mTorr. The following sublimation conditions were used: 180°C , 16 minutes for CHCA, 135°C , 7 minutes for DAN and 150°C 9 minutes for DHN to obtain a coating of 0.2 mg/cm^2 . The coating thickness measured as mass over area was obtained by weighing the slides before and after sublimation using a MS105DU Semi-Micro Balance from Mettler Toledo (Columbus, OH). The two ends of the slides were covered with a 1.25 cm width tape allowing only the middle area of 5×2.5 cm to be coated. Different coating thicknesses can be achieved using shorter or longer times of sublimation.

Pre-cleaning of new ITO microscope slides was found to be essential to obtain a uniform coating by sublimation. Fingerprints, dust particles or other contamination on the surface can result in non-homogenous coatings that can lead to flaking and peeling of the matrix during handling. In addition, efficient stirring of the sublimation flask during the sublimation process was shown to be effective in obtaining a homogenous coating.

Sample preparation

Thin sections of tissues were obtained using a Leica CM3050 cryostat (Leica Microsystems GmbH, Wetzlar, Germany). To handle sections of thickness less than 6 μm , an artist's brush with a fine tip was used to hold the sections. The placement of tissue section was carried out by a "cold press" method. Briefly, the matrix coated target was first placed in the cryostat chamber at $-20\text{ }^{\circ}\text{C}$. After the tissue section was cut, it was placed on a secondary cold Teflon coated microscope slide. The surface of the matrix pre-coated target slide was then placed on the top of the tissue section to transfer them by thaw mounting using a warm finger placed on the underside of the target slide. The slide was immediately returned to the $-20\text{ }^{\circ}\text{C}$ cryostat chamber to freeze the section and allow it to dry (approximately 5 min). The slide was then immersed in 50 mM of ammonium formate (for DAN) or 1% acetic acid (for CHCA and DHN) for 2 minutes at room temperature, then immersed into MilliQ water for 30 seconds and finally removed to dry under ambient conditions. When using DHN as matrix, because it is extremely hydrophobic, the pre-coated slide was kept frozen at least 10 minutes before placement of the tissue section. This step is necessary to introduce moisture onto the surface and increase its hydrophilicity. For experiments involving comparisons to post-coating methods, a matrix coating of 0.2 mg/cm^2 was used.

Mass Spectrometry and Data Analysis

MALDI Imaging MS was performed on a Bruker Autoflex Speed mass spectrometer in positive and negative ion reflectron modes using FlexControl 3.3 software. Approximately 100 to 200 shots/spot were acquired with a 1k Hz repetition rate Smartbeam II Nd:YAG laser. Image acquisition was carried out using FlexImaging 3.0 and MS analysis was done with FlexAnalysis 3.3.

Negative and positive mode lipid fragmentation was performed on a MALDI-LTQ-XL hybrid linear ion trap instrument (Thermo Scientific, Waltham, MA). Lipid ion fragmentation patterns were manually interpreted using the tools and standard spectra available at lipidmaps.org. The identification was carried out using the Mass Spectrometry Peak Prediction tool from the website and matching of the fragment from the LTQ. The mass tolerance was set to be ± 0.5 Dalton.

Results and discussion

Preparation of matrix pre-coated slides

Tissue sections—Treatment of tissue sections with a buffer solution greatly improves the detection level for lipids using targets prepared by sublimation.¹⁵ A modified method was utilized with matrix pre-coated slides, because the hydrophobic nature of DAN, CHCA and DHN matrices. The matrix pre-coated targets were rinsed just after placement of the tissue prior to imaging. For pre-coated DAN targets, a 50 mM ammonium formate rinse gave increased signal intensities recorded in the mass spectra of approximately 2 fold for both positive and negative ion mode (Figure 1). Similarly, rinsing targets that are coated with CHCA and DHN with 1% acetic acid gave an increase in signal intensities.

Effect of matrix coating and tissue thickness—For pre-coated targets, the tissue section thickness is an important consideration. With a constant laser output setting, we have

observed that DAN pre-coated targets (Figure 2) produce excellent mass spectra with range of section thicknesses of 3.9 μm with matrix coatings of $> 0.4 \text{ mg/cm}^2$. At 12 μm thickness, the signal intensities from the tissue were weak regardless of thickness of the matrix coating.

When analyzing tissue sections of equal thickness, the thickness of matrix layer significantly affects signal intensity with pre-coated targets. This is shown in Figure 2, where the signal intensities in the average spectra from rat kidney sections increased when the coating thickness increased from 0.088 mg/cm^2 to 0.39 mg/cm^2 and then was constant from 0.39 to 0.69 mg/cm^2 . Similar behaviors for both tissue thickness and amount of matrix were observed with CHCA and DHN (data not shown).

Comparison of matrix pre-coating and post-coating for imaging—Generally, a slightly higher laser energy is needed for samples prepared using matrix pre-coated targets to obtain equivalent signal intensities when compared to matrix post-coated procedures. Nevertheless, the spectra from tissue sections on a DAN pre-coated slide are essentially identical to that obtained from DAN post-coated tissue sections (Figure 3). Although there are peak intensity differences between the two sample preparations, the relative distribution of the peaks on the tissue sections, (i.e., the ion images), are the same as shown in the Figure 3. Since the sample prepared on pre-coated targets was treated with ammonium formate, a decrease in positive ion adducts was observed.

The utility of matrix pre-coated targets was further demonstrated by mouse brain serial sections imaged at 150, 10 and 5 μm spatial resolutions (Figure 4). At 150 μm , the result from pre-coating is essentially the same as seen in Figure 4, while at 10 and 5 μm spatial resolutions, the pre-coated targets were found to produce images with a slightly higher sharpness than the post-coated sample.

Minor artifacts in the images, such as occasional “black” pixels and striping effects seen with post- and pre-coated samples are currently being investigated although their origin is not clear at this time.

Reproducibility and robustness of pre-coated targets—Matrix pre-coated slides can be made beforehand in batches, enabling quality control assessment of each slide either optically or gravimetrically. Using targets with a uniform and fixed thickness of matrix coating, high reproducibility can be achieved, as demonstrated in Figure 5, where nine serial rat brain sections were placed separately onto three DAN pre-coated slides with the same thickness of matrix coating. Nearly identical ion images were obtained.

Stability of matrix pre-coated slides—DAN pre-coated slides are stable on storage in a vacuum desiccator. Figure 6 shows images obtained from DAN pre-coated slides that were stored for 50 days, giving essentially identical spectra as that from freshly made pre-coated slides. Nevertheless, a poorly stored slide, e.g., a slide left on the bench under ambient condition for more than 3 days (at room temperature and atmosphere) undergoes partial oxidation and changes to a brown color. The mass spectra of sections prepared from this oxidized target have a higher matrix background at m/z between 400 to 600 than the ones prepared from a target preserved under vacuum. However, this does not significantly affect the detection of peaks at m/z higher than 600.

Other matrices for pre-coated targets—We also tested CHCA and DHN pre-coated slides. CHCA pre-coated slides gives lower intensity peaks overall with tissue sections of approximately 5 μm thickness in positive ion mode, however, mass spectra from a CHCA pre-coated slide have more peaks compared with those from post-coating preparations (Supplementary Figure 1). We believe this results from the placement of the “wet” tissue

section on the matrix layer thereby allowing better extraction efficiency compared to the post-coating approach where the matrix is coated on an essentially dry tissue sections.

Another naphthalene derivative, 2,6-dihydroxynaphthalene (DHN), was shown to be amenable to target pre-coating with a tissue section of 5–8 μm thickness or less with negative mode imaging. The comparison of pre- and post-coated DHN ion images from rat kidney shows those two sample preparation methods are highly similar (Supplementary Figure 2). One advantage of using DHN as a pre-coated matrix is that this compound is very stable under ambient conditions. The spectra from a 20 day old slide preserved at ambient conditions in a clean environment gave similar images to those from a freshly prepared slide.

Conclusion

We have shown that matrix pre-coated slides can be used to image lipids at low and high spatial resolution and with performance equivalent to post coating techniques. Because of its high reproducibility and robustness, the advantages of using matrix pre-coated slides are quite significant. Pre-coated slides can be made in batches and stored for many months. The need for expensive and time consuming coating techniques during the sample preparation and analysis for a given analysis is virtually eliminated. The ease of use of pre-coated slides enables the investigator to perform the sample preparation procedure in a few easy steps, minimizing subjective variation of different personnel, decreasing costs and more rapidly producing images.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This project was supported by grants from the National Institute of General Medical Sciences (8 P41 GM103391-02 (formerly NCRR 5P41RR031461-02) and 5R01 GM058008-13) from the National Institutes of Health and the Department of Defense W81XWH-05-1-0179.

References

- (1). Caprioli RM, Farmer TB, Gile J. *Anal. Chem.* 1997; 69:4751–4760. [PubMed: 9406525]
- (2). Chaurand P, Caprioli RM. *Electrophoresis.* 2002; 23:3125–3135. [PubMed: 12298084]
- (3). Puolitaival SM, Burnum KE, Cornett DS, Caprioli RM. *J. Am. Soc. Mass Spectr.* 2008; 19:882–886.
- (4). Cornett DS, Frappier SL, Caprioli RM. *Anal. Chem.* 2008; 80:5648–5653. [PubMed: 18564854]
- (5). Hsieh Y, Chen J, Korfmacher WA. *J. Pharmacol. Toxicol. Methods.* 2007; 55:193–200. [PubMed: 16919485]
- (6). Kaletas BK, van der Wiel IM, Stauber J, Dekker LJ, Guzel C, Kros JM, Luider TM, Heeren RMA. *Proteomics.* 2009; 9:2622–2633. [PubMed: 19415667]
- (7). Zhang HY, Caprioli RM. *J. Mass Spectrom.* 1996; 31:690–692. [PubMed: 8799304]
- (8). Miliotis T, Kjellstrom S, Nilsson J, Laurell T, Edholm LE, Marko-Varga G. *Rapid Commun. Mass Sp.* 2002; 16:117–126.
- (9). Zhang X, Narcisse DA, Murray KK. *J. Am. Soc. Mass Spectr.* 2004; 15:1471–1477.
- (10). Wall DB, Berger SJ, Finch JW, Cohen SA, Richardson K, Chapman R, Drabble D, Brown J, Gostick D. *Electrophoresis.* 2002; 23:3193–3204. [PubMed: 12298091]
- (11). Grove KJ, Frappier SL, Caprioli RM. *J. Am. Soc. Mass Spectr.* 2011; 22:192–195.

- (12). Manier ML, Reyzer ML, Goh A, Dartois V, Via LE, Barry CE, Caprioli RM. *J. Am. Soc. Mass Spectr.* 2011; 22:1409–1419.
- (13). Angel PM, Spraggins JM, Baldwin HS, Caprioli R. *Anal. Chem.* 2012; 84:1557–1564. [PubMed: 22243218]

Figure 1.

Comparison of MALDI average spectra of rat kidney sections with or without a rinsing treatment for negative and positive mode with DAN pre-coated targets. The spectra were averaged from 90 locations on each section. The experimental instrument settings were identical in all experiments.

Figure 2.

The relationship between the thickness of tissue sections and the thickness of pre-coated matrix layer on signal intensity in the form of mass over area (mg/cm²). The spectra were averaged from around 90 locations on each serial section of a rat kidney. The experimental instrument settings were identical in all experiments.

Figure 3.

Comparison of images of rat kidney serial sections between matrix pre- and post-coated target at 150 μm spatial resolution (tentative identification based on previous MS/MS analyses of lipids at these masses in mouse kidney). H&E optical scan images from a serial section is shown in the first panel of the positive mode window.

Figure 4.

The comparison of imaging results of mouse brain serial sections with matrix pre- and post-coated targets at different spatial resolutions. Ion images (tentative identification based on previous MS/MS analyses of lipids at these masses in mouse brain) were collected in negative ion mode. H&E optical scanned images from a serial section are shown at the bottom of the figure.

Figure 5.

Reproducibility of imaging with matrix pre-coated target of nine serial sections of a rat brain exemplified using three lipid ions in negative ion mode (tentative identification based on previous MS/MS analyses of lipids at these masses in mouse brain). The H&E optical image from a serial section is shown in the first panel.

Figure 6.

Stability of DAN pre-coated targets demonstrated by comparison of spectra from rat kidney serial sections prepared with a freshly made target (A) and a 50 day old target preserved under vacuum (B). These average spectra were obtained from approximately 90 locations on each section. The experimental instrument settings were identical for all experiments.