Protocol

In Situ Desorption Electrospray Ionization (DESI) Analysis of Tissue Sections

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This protocol was adapted from "Desorption Electrospray Ionization: Proteomics Studies by a Method that Bridges ESI and MALDI," Chapter 6, in *Proteomics: Methods Express* (eds. O'Connor and Hames). Scion Publishing Ltd., Oxfordshire, UK, 2007.

INTRODUCTION

Desorption electrospray ionization (DESI) allows in situ analysis of biological tissues. The analysis of less abundant protein constituents within a tissue sample often requires the removal of lipid species prior to analysis, similar to the situation with matrix-assisted laser desorption/ionization (MALDI). After removal of lipid constituents, the tissue can be treated with protease to degrade proteins present in the tissue. The tryptic products can be investigated directly from the tissue using DESI. The spectra obtained feature ions of tryptic fragments from abundant proteins present in the tissue sample. The digestion is usually not complete; hence, the presence of missed cleavage sites is typical in the peptides detected. The signal is more stable for longer times than in the case of deposited samples, so the recording of mass spectrometry (MS)/MS data is simple in this case. DESI-MS is an emerging technique with great promise, but its application range is still being investigated. Therefore, the protocol for DESI-MS analysis of tissue sections presented here provides general procedures used for the applications that have been investigated so far. Optimal ion source parameters and surface types may vary depending on the application.

RELATED INFORMATION

An introduction to DESI instrumentation, methods, and applications is provided in the *CSH Protocols* article **Desorption Electrospray Ionization: Proteomics Studies by a Method that Bridges ESI and MALDI** (this issue). Protocols for **Desorption Electrospray Ionization (DESI) Analysis of Intact Proteins/Oligopeptides** and **Desorption Electrospray Ionization (DESI) Analysis of Tryptic Digests/Peptides** are also available (this issue).

Wiseman et al. (2005) analyzed adenocarcinoma tissue from human liver and determined the lipid composition at a series of positions across the tissue. In these experiments, thin frozen tissue sections were analyzed directly in the laboratory environment without additional treatment. The results from these studies indicated that the abundance of specific lipid species was enhanced in the tumor portion of the tissue. In situ analysis of unprocessed tissue is typified by the DESI mass spectrum shown in Figure 1A. In this case, an intact *Gallus gallus* (chicken) heart was analyzed using DESI. The most abundant ions present in the mass spectrum were phospholipids and other lipid species. Figure 1B shows the DESI mass spectrum recorded on a dried blood spot on the *G. gallus* heart. The analysis of the blood residue on the chicken heart showed the presence of heme and hemoglobin chains, as would be expected.

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <I>, and recipes for reagents marked with <R>.

Reagents

<I>Acetone
<R>Alkylated trypsin solution
<I>Chloroform
<I>Dichloromethane
<I>Spray solvent (e.g., methanol/H₂O or aqueous buffers)
 <I>Typical spray solvents are acidified aqueous solvents, for example, 0.1% acetic acid in H₂O.
Tissue sections (fresh or frozen)

Equipment

Mass spectrometer equipped with DESI ion source
Pipette tips
Spray chamber
Surface slides (polymethyl methacrylate [PMMA], polytetrafluoroethylene [PTFE], or glass)
Vacuum desiccator

METHOD

1. Prepare a 1:1 solution of chloroform:acetone. Thaw-mount a fresh or frozen tissue section onto a PMMA, PTFE, or glass slide, and wash three times with the chloroform:acetone solution.
2. Remove any traces of acetone by washing the sample in dichloromethane.
3. Spray deposit alkylated trypsin solution (10 µg/mL) onto the tissue section, to obtain a surface concentration of trypsin of 0.1-1 ng/mm².
4. Incubate the sample for 1 h at 35°C-40°C at 95%-100% relative humidity.
5. Dry the sample under vacuum for 3 h.
6. Expose the dried sample to DESI analysis; typical operating parameters used for in situ analysis of tissue are as follows:

Parameter	Value
Spray tip-to-surface distance	0.5-1 mm
Incident angle of spray	85°
Spray tip-to-MS inlet distance	3-5 mm
Collection angle	5°
Solvent flow rate	0.1-3 µL/min
Nebulizing gas linear velocity	300-400 m/sec
Spray high voltage	4-6 kV
Surface temperature	80°C-100°C

See *Troubleshooting*.

TROUBLESHOOTING

Problem: There are no surface-originated ions in the spectra.

[Step 6]

Solution: A possible cause of this is that the sprayed droplets and ions are not reaching the surface because of incorrect spray parameters (i.e., the volumetric flow rate is too low or there is no applied

FIGURE 1. In situ DESI analysis. (A) In situ DESI analysis of intact chicken (*G. gallus*) heart using ethanol/H₂O spray solvent at 5 μ L/min. (B) DESI mass spectrum of hemoglobin (Hgb) chains recorded on a dried blood spot on the chicken heart. (*m/z*) Mass-to-charge ratio. (Reprinted with permission, 2007 © Scion Publishing Ltd.)

high voltage) or surface charging effects. Always check the spray pattern on the surface. If the spray is not visible on a glass surface, check to ensure that the solvent syringe pump is on and that there are no blockages in the solvent delivery line that would restrict flow.

Problem: No signal is detected.

[Step 6]

Solution: There may be no spray present, or the sprayed species are being deflected by the charged surface.

Try the following:

1. Increase solvent and gas flow rates.
2. If there is no change, remove the surface and check whether the spray produces ions. Test the spray with 10 mg/mL bovine cytochrome *c* in 10 mM aqueous ammonium acetate. The test solution should give a narrow charge state distribution of the protein, with a main charge state of +7 or +8. If there are no ions, check whether the spray tip (or solvent line) is clogged. If the cytochrome *c* spectra are full of adducts, change the spray tip.

Problem: There is excessive adduct formation.

[Step 6]

Solution: This may be caused by insufficient desolvation due to the presence of large droplets or contamination of the surface. Increase the gas flow rate and/or heat the surface.

Problem: A transient signal, not suitable for MS/MS, is observed.

[Step 6]

Solution: A possible cause of this is lack of sample adhesion to the surface. The following are possible solutions:

1. Try a different surface material or a roughened surface.
2. Increase the spray tip-to-surface distance.
3. Decrease the solvent flow rate.

Problem: Results exhibit strong suppression effects and poor spectral resolution.

[Step 6]

Solution: This may be caused by an inappropriate solvent system. Change the solvent composition.

REFERENCES

Wiseman, J.M., Puolitaival, S.M., Takats, Z., Cooks, R.G., and Caprioli, R.M. 2005. Mass spectrometric profiling of intact biological tissue

by using desorption electrospray ionization. *Angew. Chem. Int. Ed. Engl.* **44**: 7094–7097.

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