Title: MALDI - OR ESI? Pros and Cons for Protein and Small Molecules

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Mass spectrometry has become a very popular technique in the analytical characterization of elements and molecules that range from inorganic, organic, and biological species. This popularity has soared in the past 15 years primarily through the development of ionization sources that can easily ionize large organic and biological molecules, intact and/or with controlled fragmentation. The two primary ionization mechanisms responsible for this capability are Matrix Assisted Laser Desorption Ionization (MALDI) and Electrospray Ionization (ESI). The development of the latter resulted in the 2002 Nobel Prize in Chemistry Engineering for John Fenn. This capability has presented a new paradigm allowing the field of proteomics to break through, with the characterization of major fractions of the proteins in a biological cell. The sensitivity, specificity, and structural characterization of available today using these techniques will be discussed with some examples in the characterization of both large and small molecules and relative merits of each technology.
MALDI- or ESI? Pros and Cons for Protein and Small Molecules

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Outline

• Typical proteomic workflow
• Comparison of electrospray versus MALDI ionization
• Issues with high-throughput analyses
• Separation strategies
• Differential profiling - ICAT
• Online or offline MS interfacing?
How does one define proteomics?

- Large-scale determination of cellular function at protein level?
- Studying the protein complement of the genome?
- Mass spectrometric identification?
- PAGE, western blotting, immunoprecipitation?
- Chip based arrays?
- Functional studies?
- Post-translational modifications?
- Qualitative/quantitative measurements to characterize processes?
- All of the above and more!!!!!!!!!!

The scientific discipline of characterizing and analyzing the proteins, protein interactions and protein modifications of an organism.
The Challenge of Proteomics
Complex Proteome(s)

- Multiple Proteins for each Gene
- Varied and fragile nature of proteins
- Quantitative and Qualitative changes of the proteome
- Structural and Functional Proteomics Studies

Courtesy: Karl Guetzow, Applied Biosystems
(1) Sample fractionation

SDS-PAGE

Excised proteins

(2) Trypsin digestion

Peptide mixture

(3) Peptide chromatography and ESI

(4) MS

(5) MS/MS

What is Mass Spectrometry?

• Belongs to a category of sizing techniques.
• Sees only ionized species, measures mass-to-charge ratios (m/z).
• Information about mass independent of the shape of the molecule.
1. Make ions: ion source
   MALDI, ES
2. Separate ions: mass analyzer
   TOF, Q
3. Detect ions: detector
4. Generate information: mass spectrum
Ion Sources make ions from sample molecules

**Electrospray ionization:**

- **Pressure** = 1 atm
- **Inner tube diameter** = 100 μm

**Sample Inlet Nozzle** (Lower Voltage)

- **Sample in solution**
- **N₂ gas**
- **High voltage applied to metal sheath (~4 kV)**

**Charged droplets**

**Partial vacuum**

- **MH²⁺
- MH⁺
- MH²⁺
Matrix Assisted Laser Desorption Ionization (MALDI)

1. Sample is mixed with matrix (X) and dried on plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules (M) are ionized by proton transfer:
   \[ XH^+ + M \rightarrow MH^+ + X. \]
Different ESI vs. MALDI characteristics on the peptide level

- better success rates with ESI on doubly charged (small?) peptides
- better success rates with MALDI on more basic (bigger?) peptides

Similar distribution was observed with the inclusion of all precursors (non-identified peptides)
Proteomics Experimental Approach

1D or 2D gel

Run gel, stain

Excise spot, destain, wash, digest, extract peptides

MALDI-TOF

Spot onto plate and mass analyze

Protein Prospector

Search all spectra against protein databases

Access database

Summarize and database results
Create Mass List from Spectrum

Process data and peak detect spectrum

Create mass list from spectrum

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Filter settings remove unwanted masses before database searching

Exclude range of peaks such as low mass matrix ions

Exclude specific containments such as keratin or trypsin
MS/MS: Tandem Mass Spectrometry

- Sample ionized
- Select mass
- Break into fragments
- Analyze fragments

Provides information about the basic structure of the compound
ICAT™ Reagent Method

Sample 1 (light)

labeled cysteines

Sample 2 (heavy)

labeled cysteines

Pool Labeled Proteins

Digest ICAT Labeled Proteins

Purify ICAT Labeled peptides on avidin

Quantitation →

MS

light heavy

550 560 570 580 m/z

100

EACDPLR ICAT

Identification

0 200 400 600 800 m/z

MS/MS
Isoelectric focusing of proteins

Treat with sinapinic acid (matrix)

MALDI MS performed directly on strip

Data acquired = pI, MW and intensity (virtual 2D gel)
Surface similar to C18 (ziptips)

70, 3757-3761

Figure 1. Mechanistic illustration detailing the synthesis of ion-pairing SPE/MALDI-MS probe.

Figure 2. Schematic illustration of on-probe sample cleanup using ion-pairing SPE/MALDI-MS.
Angiotensin I

Ion Intensity (V)

- [M+H]^+
- [M+H]^-

1 μM (200 amol)
100 nM (20 amol)
10 nM (2 amol)
Blank

m/z
900 1100 1300 1500 1700

ATMOSPHERIC PRESSURE

- sample & buffer microfluidics
- (+ HV)
- liquid junction (0 V)
- capillary probe
- separation capillary
- infusion capillary
- target coil
- repeller center (tape guide)
- rubber wheel
- propelled shaft
- interface flange
- polycarbonate block

ION SOURCE CHAMBER

PEEK liner
Protein

peptides

Enzymatic fragmentation

MS entrance

CID spectra

Protein sequence identity

Protein amino acid sequence:
MKCLLAKNTSCGLERLALTGAQALIVTQTMDISLLDAQSAPLRVVEELKIPPEGDTIPGIRLQKWENG
ECAOKKIAEKTIPAVFKIDALNENKTYLVDTDYRKKYYLFMENSAEPEQSLACQCLVR

Figeys and Pinto. Electrophoresis 2001, 22, 208-216
Liquid chromatography

Pulsed laser

Sample plate

Matrix-assisted laser desorption/ionization (MALDI)

Ion source

Mass analyser

Detector

Nozzle

Sampling cone

Electrospray ionization (ESI)

Spray needle

Reflector

TOF

Sample plate

Extraction grid

Ion trap

Collision cell

Fourier transform ion cyclotron resonance mass spectrometer (FT-MS)

Reflector

Overview

The primary objective of this study was to develop a method to digest proteins with trypsin on intact (immobilized pH gradient) IPG strips in a manner that would permit subsequent MALDI-TOF MS analysis of the peptides "on-strip." Such a procedure would enable identification of proteins separated by isoelectric focusing and bypass the second dimension SDS-PAGE, thereby saving time and tedious procedures. The analysis of intact proteins by surface MS on IPG strips has been demonstrated by Loo et al. [1] in a study where they also used cyanogen bromide cleavage to generate fragments that could be analyzed by MALDI-TOF. The chief interest in using trypsin is obviously to facilitate the identification of separated proteins by standard database searching. However, tryptic peptides are typically smaller (<3 kDa) and would tend to diffuse along and outside the gel matrix of the IPG strip. Hence, we were interested in developing a procedure that would effectively digest the proteins while preventing diffusion. We approached this problem from two directions. In the first, we excised regions of the strip that were determined to contain protein(s) of interest and subjected them to "in-gel" digestion procedures [2]. While this approach was successful and effective, to be truly useful as a rapid method, it was imperative that the proteins be digested on an intact strip. As mentioned above, the challenge was to obtain sufficient quality digestion to identify the protein while still preventing peptide diffusion. Thus, after the focusing step, all subsequent treatments were performed by spraying the reagents (enzyme as well as enhancers) onto the strip and immediately dried. The approach was evaluated using horse heart myoglobin as a standard.

Experimental procedure

Myoglobin was resuspended in IPG rehydration buffer and 7 cm, pH 5-8 strips were rehydrated overnight in passive mode. Rehydrated strips were focused using rapid "in-gel using the Bio-Rad IEF system. Focused strips were then treated with sinapinic acid for intact protein analysis or subjected to digestion. For intact protein analysis, strips were first incubated in 50% acetonitrile:0.1% TFA for 15 minutes followed by saturated sinapinic acid in the same solution for 15 minutes. Between the two incubations, the strip was dabbed with a wet Kim Wipe to remove excess mineral oil from the focusing procedure. As suggested by Loo et al. [1], 2% glycerol was added to the sinapinic acid solution to improve the integrity of the strip in the subsequent MALDI step. The strip was allowed to air-dry overnight and mounted on MALDI probes. MS was performed on an ABI Voyager DESTR in linear positive mode.

For digestion, parallel strips were rinsed briefly in d.i. water to remove excess oil, followed by a two-minute soak in 100 mM ammonium bicarbonate (AMBIC) for 15 minutes at 56°C. This was followed immediately by treatment with 55 mM iodoacetamide in 100 mM AMBIC for 10 minutes at room temperature in the dark. The strips were then washed with 100 mM AMBIC for 5 minutes. An equal amount of acetonitrile was added to the final wash and incubated for an additional 5 minutes. Following this, the strips were allowed to dry on the lab bench for 10 minutes. A 50-ng/mL solution of modified porcine trypsin (Promega) in 50 mM AMBIC was sprayed on the dried strip in short bursts while the strip was placed in a plastic rehydration tray on ice. This process was continued for approximately 30 minutes and care was taken to ensure that any excess "un-absorbed" solution was removed immediately. A little AMBIC was added to the wells of the tray not adjacent to the one containing the strip and the strip was closed and sealed before being placed in a 37°C incubator for ~14 hours. Following the digestion, a saturated solution of α-cyano matrix was added to the strip. This was allowed to dry (typically 3-4 hours) before mounting MALDI probes for MS analysis in reflector mode.

Results

The major myoglobin tryptic peptides observed with "spray" application were 522.5, 1906.8, 1815.9, 1885.0 and 2005.1. While these are not all the peptides expected from trypsin cleavage of myoglobin, they were sufficient for unambiguous identification of the protein. Interestingly, the spectral profile from digestion of an excised segment of the strip is different from that of the spray-on application: the 1606 peptide is predominant in the former while peptide intensities are more evenly distributed in the latter, reasons for which are not completely understood yet. In addition, the myoglobin tryptic peptide profile was found to be spread over 4 adjacent spectra, which adds up to 0.14 pU units. In comparison, the intact protein was spread over 3 adjacent spectra or 0.12 pU units. Thus the diffusion of the peptides can be limited significantly using this approach. The method may be suitable for applications such as protein analysis in fixed tissues or other matrices.

Comparison of trypsin digestion by "spray" method (right panel) and conventional "in-gel" digestion of a section of an IPG strip (left panel).

Zoomed images of major myoglobin tryptic peptides obtained with "spray" digestion.

Literature cited


Acknowledgements

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C60 on mesoporous silicon chip, direct MALDI without matrix, Uncalibrated.