Developments in CE/MS analysis of Proteins

Mike Knierman
Sr. Research Scientist
Eli Lilly and Co.
Career has heavily involved protein and peptide mass spectrometry

Interested in top down characterization of proteins.

Poor LC separations of larger proteins, carryover / plugging is a big problem

I see many different types of samples from pure proteins, immunoprecipitations from biological samples, to cell lysates. As such I need a universal CE method to start with for all proteins.
Why Capillary Electrophoresis?

• Separation does not require proteins to absorb on a surface
• Very small amounts of material used in separation
• Can get very high resolution separations
• At pH <3 all proteins should be positively charged and migrate to the mass spectrometer. (Universal method)
• How to interface to the mass spectrometer?
**Mass Spectrometry Overview**

### Ionization
Need to bring molecule from the liquid into the gas phase with a charge(s)

### Analysis
Need to separate the ions by mass/mass/charge

### Detection
Need to observe separated ions
Destructive or nondestructive
Dovichi CE-MS interface

- LRAP funded project to bring the technology to Lilly and develop CE/MS of proteins

**Figure 3.** Sample plug concentration profile at different time points, in the region between capillary exit and ESI emitter. Separation buffer potential: 250 V/cm, sheath-liquid potential: 100 V/cm.

Initial Implementation on Thermo orbitrap velos pro
Separation of 4 model proteins in 0.1% formic acid in water

Neutral LPA coated capillary

Operational issues

♦ Bubble formation in glass needle
♦ Very hard to flush out once formed
  • Resulted in replacing the glass needle
♦ Contamination of sheath liquid
  • If a large amount of protein was injected the excess would linger in the sheath and cause background issues.
  • Long time to bleed out before next sample.
♦ Tight space between CE and MS to adjust interface
♦ Visualization of capillary placement and nanospray
Improvements

♦ Increase diameter of glass needle to 1.6 mm OD and 1.1 mm ID.
  • Allows use of standard HPLC fittings
  • Allows up to 3 x 360 um OD capillaries to be placed in needle

♦ Second capillary is used to provide a constant back flush of sheath liquid at 20 ul/min.

Sheath liquid capillary  Separation capillary
Improvements

♦ Modified nanospray source for a better camera system
♦ Added remote electronic linear actuators for source adjustment.
♦ HPLC pump with degasser for sheath liquid helps prevent bubble formation
♦ Nanospray voltage always on. Keeping the glass needle spraying prevents from it from drying out and plugging.
♦ Neutral capillary with etched tip to get closer to nanospray tip. (Commercialized by CMP)
Innovation – CE divert valve

Divert valves are common on LC/MS systems, help protect the MS system.

Analysis state

Divert state
CE divert valve

Example - Diverted protein peak
CE divert valve - Uses

♦ Divert sample buffer peak away from nanospray emitter
♦ Divert large peaks when looking for trace analytes
♦ Divert capillary flush from nanospray emitter
  • With this ability could you used non mass spectrometry compatible buffers as a background electrolyte?
  • This would allow CE/MS to leverage a large body of previous CE work on protein separations.
  • Initial attempts were hampered by electrospray ion suppression in the buffers.
Standard Agilent micro vials need a minimum of 10 µL for injecting on the CE system. The injection volume is 0.2-0.4% of the sample (~20-40 nl).

Designed a vial insert with a sealed 10 µL pipette tip to reduce required volume to 2 µL resulting in a 5x sensitivity gain. (injecting 1-2% of the sample)

The 2 µL volume allows for many injections.
Load 2 uL sample
Then Spin to load into tip

Use Eppendorf gel loading pipette tips to remove or mix reagents with sample
Replaced triple tube CEMS source with the on-axis nano sheath source to increase sensitivity (~10x)

Method:
60 cm x 50 µm ID
CMP Tapered Cap
1 mg/ml BSA pre wash

1% Formic acid BGE
2 min preflush, 1 min post flush
30 kV for 14 min followed with 1 min flush at 30 kV

10% methanol, 0.1% formic acid sheath

2 µL sample in nanovial inserts
50 mbar 20 sec hydrodynamic injection

3000V ESI voltage

600-3200 mz
New design of CEMS source on Agilent Q-TOF

Motion controller remote

Camera

Motion controllers

Monitor
Source close up

- CE 360 um capillary
- Sheath 360 um capillary
- Vacuum Waste Line
- HPLC union
- SS HPLC tubing very wide bore
- Motion controllers
CE/MS system

HPLC pump for sheath

chiller

6550 QTOF
Example #1: Intact Antibody

Deglycosylated intact antibody

Ab

PNGase-F

145347.7 Da
Example #2: Antibody Biotinylation

Starting sample
Deglycosylated antibody & NHS-LC-biotin

Residual glycosylation
Example #2: Antibody Biotinyltion

80 min reaction time
Example #2: Antibody Biotinylation

Reduction of 80 min sample with DTT in vial
Example #2: Antibody Biotinylation

XIC for hydrolyzed biotin reagent
Neutral at low pH
Eluting at the end flush

Is a good marker to follow efficiency of clean up steps
Example #3: Abeta 1-40 Quantitation

Sensitivity

30 pg of abeta in vial

1 ug/ml
2 ng/2uL

X axis is concentration of abeta, but only need 2uL in vial

Only 1/100 of the sample is actually injected into the capillary
Example #4: Recombinant Protein

Expected fully reduced protein

Calc MW no disulfide = 20405.96 Da

20406.06 Da
Example #4: Recombinant Protein

Monomer - 20404.66 Da
Dimer - 40809.43 Da
Calc MW 1 disulfide = 20403.96 Da
Example #4: Recombinant Protein

Calc MW 1 disulfide = 20403.96 Da
Example #4: Recombinant Protein

Both peaks are resulting from a single disulfide bond formation

Hydrodynamic effect

Coeluting dimer

Same mass

Only by the combination of CE and MS can we quickly see this difference
Example #4: Recombinant Protein

Calc MW no disulfide = 20405.96 Da

Reduction with DTT

20406.50 Da
Mass spectrum and ion mobility spectrum of the ubiquitin peak in CE showing different conformers in the peaks at 1224 $^+$ and 1428 $^+$.
Sciex cESI system

- Electrospray off end of the separation capillary
- Neutral coated capillary available
- Nanovial 3-5 uL sample
New instrument in CEMS

♦ 908 Devices zipchip

- Based on technology out of Michael Ramsey’s lab at University of North Carolina – Chapel Hill
- Very fast CEMS runs- proteins in 3 min.
- Quick setup
Zipchip Separation of 4 Proteins

Zipchip HR on Orbitrap velos pro

Isotachophoretic loading

Ubiquitin

Lysozyme

Carbonic Anhydrase

Alpha-Synuclein

Relative Abundance

Time (min)

RT: 0.00 - 3.03

NL: 6.40E6
TIC MS
082916_4mix_1
0k_1-
5kV_lower_Ctra
p_pressure_100
mM_AmAc_08
Summary

♦ CE/MS of proteins is sensitive
♦ Low carry over of proteins observed for neutral capillary
♦ Very small amounts of sample are consumed for a CE/MS run
♦ CE/MS is amenable for intact and denatured proteins
♦ The nanosheath CE/MS system is very robust, weeks of runtime with same nanospray needle and capillary
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