Top Down Proteomics Using Online Polymer Reversed Phase (PLRP) Nanocapillary-LC Coupled Fourier Transform Mass Spectrometry

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OVERVIEW
In comparison to Bottom Up proteomics, the growing field of Top Down proteomics is impeded for high throughput, due partly to the lack of comparable studies in sample preparation and online separation methods.

Here, we evaluate the performance of Polymer Reversed Phase (PLRP) for online nano-LC coupled Top Down analysis in combination with pre-fractionation by gel-eluted liquid fraction entrapment electrophoresis (GELFEE).

RESULTS

Recovery and Resolution

<table>
<thead>
<tr>
<th>Sample</th>
<th>C4</th>
<th>PRLPS</th>
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<tr>
<td>0.3 pM</td>
<td>1.0 µL</td>
<td>1.0 µL</td>
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Chromatograms of RPLC separation of a seven protein standards mixture with varying concentrations, using C4 (blue) and PRLPS (red) 75 µm I.D. x 100 mm, 300 Å, 5 µm stationary phase (analytical) columns. Standards used were: 1. Ubiquitin; 2. Cytochrome c; 3. α-lactalbumin; 4. Myoglobin; 5. α-casein; 6. Carbonic Anhydrase; 7. Ovalbumin. PRLPS provides reduced peak widths and higher recovery leading to increased chromatographic resolution and MS sensitivity.

PLRPS Pore Size and Retention

Separation of six protein standards ranging from low mass to medium high mass were conducted on nano-LC using varying pore size. Chromatograms show inverse relationship between pore size and retention factor. These figures show that 1000 Å is the optimal pore size for both medium (30-40kDa) and high MW proteins (100-500kDa) in terms of resolution. In addition, recovery was observed to be inversely proportional to pore size. Protein standards used were: Cytochrome C; 2. α-lactalbumin; 3. Myoglobin; 4. Carbonic anhydrase; 5. Ovalbumin; 6. Bovine serum albumin.

PLRPS and High Mass Detection

Ion trap charge state distribution profiles for BSA fractionated online using PLRPS (300 Å) from six protein standard mixture with increase in number of micro scans. The S/N intensity increases with micro scans.

PLRPS and High Mass Identification

Shown here is a fragmentation spectrum from single FT scan obtained during the online nano-LC MS/MS of six protein standards mixture. Monoisotopic fragment masses were obtained from this scan using XIC and searched against a standard protein database consisting of 7365 unique protein forms. The search result showed 23 fragments matching to BSA with mass error of less than 5 ppm.

CONCLUSIONS
- PLRPS provides improved resolution and recovery over C4
- 1000 Å was the optimal pore size for resolution
- Coupling GELFEE with PLRPS enables nano-LC-MS/MS analysis of complex proteome mixtures
- The standardized methods will be applied for the identification and characterization of proteins from other proteomes such as the secretome of human globlastoma cell lines.

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