Microchip Electrophoresis Glycoprotein Separation

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Glycoproteins

- A class of proteins which have carbohydrate groups attached to the polypeptide chain

- Secretory and membrane proteins are post-translationally modified with sugar-chains called Glycans

- Essential for the stability and function

- Functions:
  - Enzymatic activity
  - Receptor binding
O-Glycans

- Protein can be modified with different sequence of carbohydrates
- N-Glycans and O-Glycans are most common in Eukaryotic systems
- O-Glycans are assembled one sugar at a time
N-Glycans

- Attached to asparagine residue of a protein

- Have common biosynthesis pathway reflected in structure of their core

- Two types:
  - High Mannose
  - Complex Glycan
Alternative techniques

- **MS (MALDI/ESI TOF)**
  - Label free, Fast and high throughput
  - Not able to separate structural isomers

- **LC (HPLC, DEAE/diethylaminoethyl)**
  - Separate structural isomers
  - Slow and Need for a standards sample

- **Capillary electrophoresis (CE)**
  - Fast and high throughput
  - Need for a standards sample
Capillary electrophoresis

https://www.youtube.com/watch?v=nBIE6aiHRok
Microchip Electrophoresis

- CE combined with sample, buffer, waste and possibly further analytical tools (essentially a probe)
- Automation possible
- Better separation performance for same V

Microchip Electrophoresis-Components and General Workflow

- Injection
  - mechanism
- Channel
  - Bare-fused silica vs. coated
  - Length, geometry & voltage
- Detection
  - Optical
  - MS
Microchip Electrophoresis-Injection

- Problem: Different electrophoretic mobility of diff. species
- Solution: “Pinched” or “Double T injection”
- Establish flow from sample reservoir to sample waste prior to injection

![Diagram of Microchip Electrophoresis-Injection]

“pinched” injection

<table>
<thead>
<tr>
<th></th>
<th>Sample</th>
<th>Buffer</th>
<th>Waste</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading (b)</td>
<td>0</td>
<td>0.22</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>Injection (c)</td>
<td>0</td>
<td>0.22</td>
<td>0.6</td>
<td>4-6</td>
</tr>
<tr>
<td>Analysis (d)</td>
<td>0.4</td>
<td>0.22</td>
<td>0.6</td>
<td>4-6</td>
</tr>
</tbody>
</table>

“Double T” injection

Microchip Electrophoresis-Channel Surface

- Bare-fused silica or coated?
- Problem: electro-osmotic effect
  - Increased separation time for -ve derivatised glycans
  - Local inhomogeneity can lead to asymmetric/distorted peaks
- Solution: Coat the surface w/ single layer of polymer
- Immobilised layer, does not move towards cathode
- Problem: to fit long channels compactly
- Racetrack Effect: Molecules at inner wall have shorter tracks and experience stronger field
- Increase radius of curvature by using spiral channels or tapering at the turning points

Microchip Electrophoresis-Channel Geometry

- \( f = \frac{v}{j_{\text{eq}} V t \theta} \)

--- = Equipotential Field Lines

Buffer waste → detect
Sample → analysis

Anal. Chem. 2012, 84 (8), 3621–3627
Anal. Chem. 1998, 70 (18), 3781–3789
Anal. Chem. 2000, 72 (23), 5814–5819
Microchip Electrophoresis-Detection

- Optical vs connection to MS
- Optical
  - Cheaper, but require standard to elucidate glycan structure
  - Fluorescent tag needed
  - Excitation Source: Argon laser vs. blue LED
  - Price vs Sensitivity
- MS: problems with flow rate, gives some structural information
Microchip Electrophoresis-Voltage

- Separation Efficiency
- Time
- Heating

\[
\left( \frac{N}{t} \right)_{\text{max}} = \frac{\mu_i^2 E^2}{2D_i + \mu_i E (\sigma_{id}^2 / L)}
\]

Sample Preparation

Denaturation → Release of glycans → Labelling
Sample Preparation-Release of Glycans

- Denaturation: By β-mercaptoethanol, SDS and high temp.
- Release of Glycan: Enzymatic, PNGase-F most common

## Sample Preparation-Derivatisation

- Problem: Some glycans are neutral and not fluorescent
- APTS label
- Effect of labelling on glycan profile
- Choice of reductant (NaCNBH$_3$ vs 2-picoline borane)

### Sample Preparation

<table>
<thead>
<tr>
<th>Temperature</th>
<th>APTS Concentration</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C; overnight</td>
<td>10 mM APTS</td>
<td>50°C; 2 hours</td>
</tr>
</tbody>
</table>

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Sample Preparation—Overall Time and Efforts

- Traditionally done in flasks, long time for glycans release, laborious
- Possibility to perform with PCR thermocycler programmes within a few hours

**Denaturation**
- 60 min, 60°C
- 10 min, 95°C & 5°C

**Release of glycans**
- 18 h, 37°C
- 10 min, 37°C

**Labelling**
- 2 h, 50°C

Microchip Electrophoresis-Data Analyses

- Profiling: Spectra as “barcode” vs thorough structural elucidation
- Compare with large set of pre-obtained data

J. Proteome Res. 2013, 12 (10), 4490-4496
Point of Care
Definition and challenges

Centrifuge
  Serum Extracting

Thermal cycler
  Denaturation of Proteins at 95C
  Deglycosylation at 37C
  Incubation for dye labeling at 55C

Capillary electrophoresis
  Separation of the Glycan
  Signal detection
Lab on chip

Reference


Thank you for your attention