FT-ICR MS in the analysis of IgA Nephropathy

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Finnigan LTQ FT-ICR Mass Spectrometer

- Ion Trap based
  Fourier Transform-Ion Cyclotron Mass Spectrometer
- FT-ICR @ LC timescale
- High Mass Accuracy
- High Mass Resolution

Linear Ion Trap – FTICR Hybrid

2D Ion Trap

ICR Cell

7 T Activity Shielded
Superconducting Magnet
Finnigan LTQ FT-MS System

Introduced 2003

ECD and IRMPD with the Finnigan LTQ FT

Linear Ion Trap MS
- MS, MS/MS and MS^n Analysis by Collision Induced Dissociation

FTICR MS
- Electron Capture Dissociation (ECD)
- Infra-Red Multiphoton Dissociation (IRMPD)

NHMFL 9.4T ESI FT-ICR MS (1995)

Alan Marshall Group
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National High Magnetic Field Laboratory,
Florida State University
IgA1 vs. IgA2

O-glycans  N-glycans

(P) V (P) S  T  P  P  T  P  S  P  S  T  P  P  T  P  S  P  S  C  IgA1

(P) V (P) --------------------------  P  P  P  C  IgA2

Hinge Region

O-glycosylation

(NeuAc) α2,3
Gal α1,3
GalNAc ----(NeuAc) α2,6
Ser/Thr

Galactose Deficient IgA1
IgA Immunodeposits & Circulating Immune Complexes

(NeuAc)

| GalNAc ----(NeuAc)
| Ser/Thr

• Bypasses liver & deposits in kidney

Questions concerning IgA1 Hinge Region O-glycosylation

P  V  P  S  T  P  P  T  P  S  P  S  T  P  P  T  P  S  P  S  C

• Heterogeneity of O-glycosylation of IgA1

• Sites of O-glycan attachment
  (N-terminal sequencing methods)

• Gal deficiency in IgAN: randomly or at specific sites
  (Glycan specific lectins: jacalin, HAA)

Galactose Deficient O-linked Glycans
Sialic Acid removed

Gal
\| GalNAc
\| Ser/Thr

\[ \text{GalNAc} \quad \text{GalNAc} \quad \text{GalNAc} \]
\| Ser/Thr
\| Ser/Thr

ESI FT-ICR MS of (Mce) IgA1 HR isolated from trypsin-pepsin-thermolysin digest

HYTNPSQDVTPCVPSTPSTPSTPSTPSPSCCHPRL

FT-ICR MS

\[ \text{m/z} = \text{Gal} \quad \text{GalNAc} \quad [\text{M} + 3\text{H}]^{3+} \quad [\text{M} + 2\text{H}]^{2+} \quad [\text{M} - 3\text{H}]^{3+} \quad [\text{M} - 2\text{H}]^{2+} \]

Quadrupole + SWIFT Isolated

Figure 4
De-glycosylated IgA1 Hinge Region

ESI FT-ICR MS
neuraminidase treated

neuraminidase + O-glycanase treated

de-glycosylated HR peptide

Confirmed IgA1 Hinge Region sequence

Confirmed IgA1 Hinge Region sequence

GlycoMod assigned O-glycans

GlycoMod assigned O-glycans
How do Peptides Cleave?

Slow heating methods

IRMPD $b_{n-1}$

Electron radical chemistry

How do Peptides With PTMs Cleave?

IRMPD $b_{n-1}$
Can We Locate O-glycosylation Sites?

VTVCPVPSSTTPSSTPPSTTPSPSCCHPRL

\[ \text{electron capture dissociation} \]

\[ R_1 \text{O} \quad H_2N-\text{C} \cdots \text{C} \cdots -\text{N} \text{-C} \text{-C} \text{-OH} \]

\[ \text{AI-ECD} = \text{Gal} \quad \text{GalNAc} \]

\[ [\text{M} + 3\text{H}]^{3+} \quad [\text{M} + 2\text{H}]^{2+} \]

\[ \times 40 \quad \times 2 \]

\[ \times 50 \quad \times 2 \]

\[ \text{m/z} 1200 \quad 1000 \quad 1400 \quad 1200 \quad 1600 \quad 2000 \quad 2200 \quad 2400 \]
Can We Locate O-glycosylation Sites?

Profiling IgA1 glycoforms:
IgA1 Hinge Region
Specific Proteases

4 GalNAc / 4 Gal
4 GalNAc / 3 Gal
5 GalNAc / 4 Gal
Micro scale-LC Fraction Collection

Chip-based ESI source
NanoMate by Advion

- 20x20 array of 8 µm nozzles
- ~300 nl/min flow
- Sample as low as 1 µl from 3 total
- 3 µl can produce ~30-40 min spray
Myeloma IgA1 Hinge Region O-glycans

IgA1 (Mce) Hinge Region O-glycans

LC-MS IgA1 (Gou) O-glycans
FT-ICR MS for the diagnosis IgA Nephropathy?

- IgAN patients
  - Severe symptoms
  - Moderate symptoms
- Non-IgA related glomerulonephritis
- Normal healthy controls

O-glycans  N-glycans

IgA1 N-glycans

Are they altered in IgAN?