MALDI-TOF MS Application

Analysis of an Intact G-Protein Coupled Receptor by MALDI-TOF Mass Spectrometry: Molecular Heterogeneity of the Tachykinin NK-1 Receptor


• Tachykinins: peptides with common C-terminal amino acid sequence Phe-X-Gly-Leu-Met-NH2
• NK: NeuroKinin
• Tachykinin NK1 receptors are found in the brain
• Are targeted in the treatment of neurological disorders
• Tachykinin may have a modulatory role in testicular function
Signal Transducing Receptors

• Plasma membrane proteins:
  – bind specific extracellular molecules (hormones, neurotransmitters, etc)
  – Transmit a signal to the cell interior
  – Signal elicits response

Families of Membrane-bound Signal-Transducing Receptors

• Ion-channel linked receptors
• Enzyme-linked receptors
• G protein-linked receptors
G-protein linked Receptors

- 7 transmembrane helices
- G proteins
  - Guanine nucleotides binding proteins
  - Molecular switches activated by binding of GTP
  - Inactivated when GTP is hydrolyzed to GDP
  - Heterotrimers
    - $\alpha$ (45kDa), $\beta$ (35 kD), $\gamma$ (8 kD)
Regulators of GTP hydrolysis (RGS)

• Bind to the active G protein and accelerate the rate of GTP hydrolysis
• G proteins on their own are very poor GTPases
• The active GTP-bound state activates many downstream effectors before RGS bind and the signal is switched off

Physiological responses triggered by G protein-linked receptors

• Vision
• Smell
• Stress response

• The human genome contains 1000 different genes for these receptors and genes for different $\alpha$, $\beta$ and $\gamma$ subunits of G proteins
Analytical Challenges

• Difficult to crystallize
• Too large for NMR analysis
• Inherent heterogeneity during their genetic expression due to splicing and posttranslational modification
Analysis of an Intact G-protein Coupled Receptor by MALDI-TOF MS: Molecular Herogeneity of the Tachykinin NK-1 Receptor

- FLAG M2 and His-tagged NK-1 receptor Cloned pTEJ8 vector Expressed in CHO-K1 cells with a
  - Chinese Hamster ovarian cells
  - Mw of FLAG M2 + Met: 1303.4
- Affinity Purification on cobalt and nickel beads (binding to C-terminal (His)_6-tag end)
- SDS-PAGE and Western Blot Analysis
- NK-1 receptor deglycosylation with N-glycosidase F
- Expression in the presence of tunicamycin to inhibit glycosilation
- Trypsin digest
- Chymotrypsin digest
- CNBr digest
- Edman sequencing

Results

- SDS-PAGE
  - apparent molecular weight Mr between: 55000-66000
  - Additional bands around ~45,000
  - Theoretical Mw of FLAG and His-tagged NK1 receptor: 48,228
  - Broad diffuse band proteins bands observed before the membrane proteins
- Western Blot against His-tag and FLAG tag
  - Mr ~ 55000-66000
  - Lower Mr species ~ 45000-50000
- Results of samples treated with N-glycosidase and enzymes to remove both O and N-linked carbohydrates
  - Mr ~ 50000
MALDI-TOF Results

- Intact receptor: 40-50 samples prepared in different conditions
- Fig 4A: glycosylated receptor
  - Different species
  - Most abundant: ~Mr~ 56000-58000 (as single, double and triple charges ions): glycosylated (G) receptor (confirmed by western blot)
  - Less abundant D (deglycosylated): M/z of 48,468: similar to mass predicted by cDNA sequence without posttranslational modification
  - Another species observed at ~112,300-114,000: dimer of G?

MALDI-TOF Results

- Fig 4C: N-glycosidase-treated sample
  - Most abundant ions: m/z 48,856 and 47,571
- Fig 4B: tunicamycin-treated cells
  - O-glycosylated form of the receptor observed (m/z 53,750-55,650)
Homework

• Provide a possible justification for the following observation:
  – Dimer of G observed by MALDI-TOF was not observed by SDS-PAGE
  – Degradation of the receptor as suggested by ions with lower m/z (500-20000)

• Summarize and discuss major results of the article: CE-Microreactor-CE-MS/MS for Protein Analysis, Schenherr et al., Anal. Chem. 2007, 79, 2230-2238