Study of non-covalent protein complexes of 20-204 kDa using a Top-Down approach on a hybrid LTQ-Orbitrap mass spectrometer

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1. Overview - Introduction

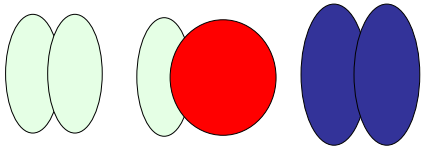
Overview

- We explored the ability of the LTQ-Orbitrap to analyze non-covalent protein complexes.
- We determined accurate mass of protein complexes and their protein subunits covering a mass range of 13-240kDa.
- Top-down analysis of the protein complexes and their subunits determined post-translational modifications and truncated isoforms.

Introduction

- Mass spectrometry has become a powerful complementary technique for the structural characterization of proteins [1].
- The mass spectrum of an intact protein defines the native primary sequence of the gene product and its heterogeneity.
- Top-down fragmentation analysis of protein and protein complexes provides useful information about sites of post-translational modifications [2, 3].
- Three purified protein complexes from the enteropathogenic *E.coli* (EPEC) were analyzed on LTQ-Orbitrap.

CesAB Homodimer	[CesAB][EspA] Heterodimer	SecA Homodimer
M_r 27821.52 kDa	M_r 34961.28 kDa	M_r 204756.36kDa



Bibliography

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2. Methods

Purified protein Complexes

Homodimer or Heterodimer

Denatured (Organic solvent) or **Native** (Ammonium Acetate) or

nano-ESI infusion

Flow rate of 200-400 nl/min with a 50μl syringe connected with a stainless steel emitter (30μm ID).

MS

Molecular Mass analysis
Protein complex stoichiometry
Isoforms/PTMs

MS/MS

Protein/complex characterization
Isoforms/PTMs

Data Acquisition:
Xcalibur 2.0.7 (Thermo Scientific)

MS Spectra Deconvolution:
Xrtract (Thermo Scientific)
ProMass 2.5 (Novartia-Thermo Scientific)

MS/MS Spectra annotation:
ProSightPTM 2.0 (Thermo Scientific)
Manually

3. Results - Conclusions

CesAB Monomer

DENATURED

MS spectrum in Orbitrap — CesAB#2 +9 → +22

Deconvoluted spectrum using Xtract — 12254.634 (-1658.35); 13439.109 (-472.73)

Deconvoluted MS/MS spectrum 791.48m/z, z=17 — CesAB#2

Determined protein mass

CesAB#1	13911.880 kDa (predicted)
CesAB#2	13439.109 kDa (Δm 7ppm)
CesAB#3	12254.634 kDa (Δm 13ppm)

Determined modifications/isoforms

#1 M- aa sequence -MNQRNRNNRKIV
#2 aa sequence -MNQRNRNNR
#3 aa sequence

➢partial N-terminal acetylation was also observed

CesAB Homodimer - CesAB/EspA Heterodimer

NATIVE

MS spectrum in Orbitrap — CesAB#2 +9 → +15; CesAB#2 +4 → +8; [CesAB#2]$_2$ +9 → +11; EspA#2 +10 → +23; EspA#2 +7 → +9; [CesAB#2][EspA#2]$_2$ +10 → +12

Deconvoluted spectrum using Xtract — 12254.629; 25693.803; 26878.344

Deconvoluted MS/MS spectra of Homodimer — CID 10, CID 15, CID 25, CID 35, CID 45, CID 55

Deconvoluted MS/MS spectra of Heterodimer — 12254.579; 33174.054; 34358.661 (-18.892); 20918.649; 13439.177; CID 35, CID 45, CID 55, CID 65

Determined protein mass/modifications/isoforms

[CesAB#1]$_2$	13911.880 kDa (predicted)
[CesAB#2]$_2$	26878.344 kDa (Δm 5ppm)
[CesAB#2][CesAB#3]	26878.344 kDa (Δm 4ppm)

➢partial N-terminal acetylation was also observed

Collision energy dependant complex dissociation

Determined protein mass/modifications/isoforms

EspA#1	21068.530 kDa (predicted)
EspA#2	20918.638 kDa (Δm 33ppm)
[CesAB#2][EspA#2]	34358.661 kDa (Δm 6ppm)
[CesAB#2][EspA#2]	33174.054 kDa (Δm 3ppm)

#1 M- aa sequence
#2 aa sequence

SecA Homodimer

MS spectrum in Ion-Trap — SecA +53 → +125; [SecA]$_2$ +56 → +164

NATIVE — Deconvoluted Spectrum using ProMass

DENATURED — Deconvoluted Spectrum using ProMass

SecA	102378.18 kDa (Predicted)
SecA	102434.40 kDa (Δm 548ppm)
[SecA]$_2$	204836.90 kDa (Δm 390ppm)