Combined Top-Down, Middle-Down and Bottom-Up Analysis of Apo A1 and PTGDS Isolated From Human CSF Using ETD-Linear Ion Trap and FT-Mass Spectrometry

Roger G. Biringer[1], Zhiqi Hao[1], Mike Harrington[2], and Andreas F. R. Hühmer[1] ● [1]Thermo Electron, San Jose, CA, [2]Huntington Medical Research Institutes, Pasadena, CA, USA

Overview

Purpose: To use complementary top-down, middle-down and bottom-up LC-MS methodologies for the purpose of identifying post-translational modifications in Human Apolipoprotein A1 (Apo A1) and Human Prostaglandin-H2 D-isomerase (PTGDS) isolated from CSF.

Methods: Infusion and column-based LC-MS methods were used to identify isoforms of Apo A1 and PTGDS.

Results: Numerous isoforms were observed and several positively identified, including an oxidized form of PTGDS and oxidized, glycated, and polymorphic forms of ApoA1.

Introduction

Isoform concentrations for two CSF proteins, Apolipoprotein A1 (Apo A1) and prostaglandin D synthase (PTGDS) have been shown to be dramatically altered in the CSF obtained from individuals with Alzheimer's disease (AD) and migraineurs respectively[1]. The goal of this study was to determine the types and sequence locations of relevant post-translational modifications (PTMs) on each of these proteins as a first step in understanding isoform variations in the disease state. Commercially available Apo A1, and Apo A1 and PTGDS isolated from human CSF were evaluated. LC-MS top-down methods as well as analysis of enzyme digests with conventional collision induced dissociation (CID) and electron transfer dissociation (ETD) were employed. The complimentary information of the top-down, middle-down, and bottom-up approaches is exploited for identification and characterization of several PTMs in Apo A1 and PTGDS.

Methods

Overview: Commercially available human Apo A1 (Calbiochem) was introduced into either a Finnigan™ LTQ™ or Finnigan LTQ FT™ mass spectrometer (both Thermo Electron) directly by infusion. Proteolytic digests of 1) commercially available human Apo A1, 2) Apo A1 and PTGDS isolated from Human CSF, and 3) whole human CSF were introduced using standard LC-MS methods.

Infusion Experiments: A Finnigan ProteomeX LTQ and a Finnigan LTQ FT equipped with an experimental electrosonic spray device (ESSI) were used to evaluate samples of Human Apo A1. Samples were infused as 10 pmol/µL at 5 µL/min.

LC-MS Experiments: A Finnigan ProteomeX LTQ and an LTQ modified for ETD were used to evaluate proteolytic digests of Human Apo A1. Chromatography was accomplished with a 200 µm x 100 mm proprietary monolithic column using a 0-80% gradient over 20 minutes or a 150 µm x 100 mm C-18 (Micro-Tech Scientific) using a 0-60% gradient over 30 minutes (A: 0.1% formic acid; B: 0.1% formic acid in 100% acetonitrile). Typical flow rates were 2 µL/min for monolithic columns and 1 µL/min for C-18 columns.

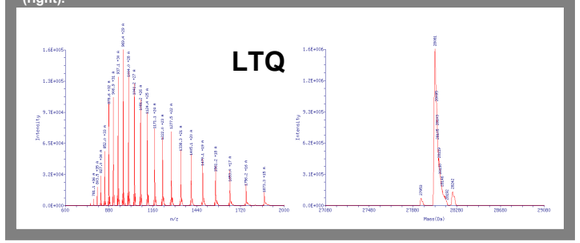
Isolation of Apo A1 and PTGDS from 1D SDS gels: Ethanol-precipitated proteins from whole CSF were fractionated on 1D SDS PAGE gels. The band corresponding to combined Apo A1 and PTGDS proteins was cut from the gel and the proteins were electroeluted.

Digestion conditions: Proteins were reduced and carboxyamidomethylated. Solutions were prepared in 100 mM ammonium bicarbonate, pH 8 and digested with either LysC, ArgC modified trypsin or GluC (all Roche) at 37 °C overnight. The pH was then reduced to 3 using small amounts of glacial formic acid and residual ammonium bicarbonate removed with a SpeedVac™ (Thermo Electron).

Data Analysis: Whole protein data was evaluated with ProMass Deconvolution™ 2.0 for Xcalibur™ (Thermo Electron). Protein digests were evaluated with BioWorks™ 3.2 (Thermo Electron) using a SwissProt Human database and the same database modified to include all reported Apo A1 mutations and polymorphisms[2].

Results: Top-Down

FIGURE 1. Mass spectrum of commercially available Apo A1 using a linear ion trap mass spectrometer in a top-down method showing a completely resolved charges state envelope for several protein isoforms (left). ProMass Deconvolution 2.0 analysis of the infusion data confirm the presence of several protein isoforms (right).



- Deconvolution of LTQ infusion data reveals the identities of charge states and 6-7 distinct isoforms.

FIGURE 2. Zoom scan spectrum of an infusion of commercially available ApoA1 on the FTMS instrument. Six major isoforms for the +31 charge state are depicted. Mass differences between isoforms were calculated from the data given in Table 1.

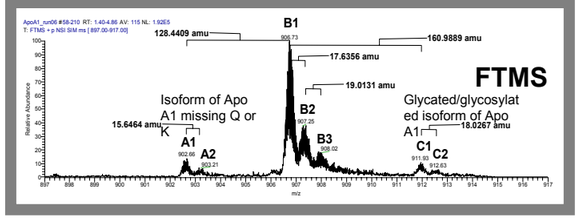


Table 1. Synopsis of zoom scan data of Apo A1 protein analysis on the FTMS instrument. Data represents the average obtained from three charge states. SD is the standard deviation of the averaged results. The peak area data is given as percentages.

Peak	Mass (amu) average	Mass (amu) SD	Peak Area (rel%) AvsBvsC	Peak Area (rel%) 1vs2vs3
A1	27950.3218	0.5728	10	
A2	27965.9682	0.6039		
B1	28078.7627	0.0137		11
B2	28096.3983	0.5757	80	25
B3	28115.4114	2.1709		64
C1	28239.7516	0.0148	10	
C2	28257.7783	0.1827		

- Results of infusion experiments on the Finnigan LTQ FT instrument reveal more detail and clearly show the presence of at least 7 distinct isoforms of which the low mass isoform is consistent with the deletion of Q or K, both of which are known polymorphic forms of Apo A1[2, 3].

Results: Sequence Coverage

FIGURE 3. Combined Coverage obtained for commercially available Apo A1 from both ETD and CID experiments. Blue = CID only, Red = ETD only, Purple = both ETD and CID, Black = not identified.

Apo A1 LysC and ArgC digests (96.7% coverage)

DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTF
SKLREQLGPVTQEFWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDFQKKWQEEM
ELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDE
LRQRLAARLEALKENGGARLAEYHAKATEHLSTLSEKAKPALEDLRQGLLPVLESFKV
SFLSALEEYTKKLNTQ

Apo A1 LysC , ArgC and GluC digests (99.2% coverage)

DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTF
SKLREQLGPVTQEFWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDFQKKWQEEM
ELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDE
LRQRLAARLEALKENGGARLAEYHAKATEHLSTLSEKAKPALEDLRQGLLPVLESFKV
SFLSALEEYTKKLNTQ

- Combined ETD/CID analyses provide excellent coverage.
- GluC peptides provide additional sequence information.

FIGURE 4. Combined Coverage for Apo A1 isolated from Human CSF and in whole Human CSF digests (LysC, ArgC and trypsin) obtained from both ETD and CID experiments. Blue = CID only, Red = ETD only, Purple = both ETD and CID, Black = not identified.

Apo A1 isolated from 1D SDS PAGE gels (63.0% coverage)

DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTF
SKLREQLGPVTQEFWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDFQKKWQEEM
ELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDE
LRQRLAARLEALKENGGARLAEYHAKATEHLSTLSEKAKPALEDLRQGLLPVLESFKV
SFLSALEEYTKKLNTQ

Apo A1 observed in Human whole CSF digests (70.4% coverage)

DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTF
SKLREQLGPVTQEFWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDFQKKWQEEM
ELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDE
LRQRLAARLEALKENGGARLAEYHAKATEHLSTLSEKAKPALEDLRQGLLPVLESFKV
SFLSALEEYTKKLNTQ

FIGURE 5. Combined Coverage for PTGDS isolated from Human CSF and in whole Human CSF digests (LysC, ArgC and trypsin) obtained from both ETD and CID experiments. Blue = CID only, Red = ETD only, Purple = both ETD and CID, Black = not identified.

PTGDS isolated from 1D SDS PAGE gels (41.1% coverage)

APEAQVSVQPNFQQDKFLGRWFSAGLASNSSWLREKKAALSMCKSVVAPATDGGLNL
TSTFLRKNQCETRTMLLQPAGSLGSYSYRSPHWGSTYSVSVVETDYDQYALLYSQGSK
GPGEDFRMATLYSRTQTPRAELKEKFTAFCKAQGFTEDTIVFLPQTDKCMTEQ

PTGDS observed in Human whole CSF digests (61.3% coverage)

APEAQVSVQPNFQQDKFLGRWFSAGLASNSSWLREKKAALSMCKSVVAPATDGGLNL
TSTFLRKNQCETRTMLLQPAGSLGSYSYRSPHWGSTYSVSVVETDYDQYALLYSQGSK
GPGEDFRMATLYSRTQTPRAELKEKFTAFCKAQGFTEDTIVFLPQTDKCMTEQ

Results: Posttranslational Modifications

Table 2. Oxidized methionine residues of ApoA1 and PTGDS found in whole Human CSF and protein isolated from Human CSF.

	ApoA1	PTGDS
Isolated from Gel	M-112	M-72
Whole CSF	-	M-72

Table 3. Protein database search results of proteolyzed commercially available Apo A1 protein isoforms. Peptides supporting the presence of both modified and corresponding unmodified peptides are shown. Oxidatively modified methionine residues are highlighted in red. Amino acid residue polymorphism of a missing amino acid residue is indicated with an underlined blank space. Observed propeptide portion of incompletely processed protein is given in blue.

protease	peptide	Charge	XCorr	Modified residue
LysC	ETEGLRQEMSKDLEEVK	2	3.28	M-84
LysC	ETEGLRQEMSK	2	2.99	none
LysC	KWQEEMELYRQK	2	3.94	M-112
LysC	KWQEEMELYRQK	2	4.26	none
GluC	TEGLRQEMSKDLEE	2	3.35	M-84
GluC	EGLRQEMSKDLEE	2	2.81	M-84
GluC	VKAKVQPYLDDFQKKWQEEME	3	4.97	M-112
GluC	VKAKVQPYLDDFQKKWQEEME	2	4.79	none
GluC	MELYRQKVEPLRAELQE	3	4.68	M-112
GluC	MELYRQKVEPLRAE	2	3.71	none
protease	peptide	Charge	XCorr	Missing residue
GluC	VKAKVQPYLDDFQK_WQEEME	3	6.21	K-107
GluC	VKAKVQPYLDDFQKKWQEEME	2	4.95	none
GluC	EVKAKVQPYLDDFQK_WQEEME	3	5.22	K-107
GluC	EVKAKVQPYLDDFQKKWQEEME	3	5.06	none
GluC	YTKKLTQ-	2	2.91	none
ArgC	VSFLSALEEYTKKLNTQ-	2	4.60	none
ArgC	VSFLSALEEYTKKLNT-	3	4.23	Q-243
protease	peptide	Charge	XCorr	Additional residue
LysC	HFWQQDEPPQSPWDRVK	3	4.18	propeptide

- Peptides containing oxidized methionines and their non oxidized counterparts were observed, indicating that isoforms with either or both oxidized M-84 and M-112 residues are present in the protein mixture together with non oxidized forms of the Apo A1 protein.
- Apo A1 N-terminal peptides containing and missing the terminal Q residue were observed, indicating that the low mass isoform observed in the Top-Down experiments has either a K deletion at amino acid residue 107 or is missing the terminal Q.

Conclusions

- A combination of both top-down and bottom-up mass spectrometry methodologies provide complimentary information required for protein isoform identification.
- The polymorphic A2 isoform depicted in Table 1 most likely represents a methionine-oxidized (M-112) Marburg/Munster-2 isoform where K-107 is missing and A1 an isoform missing the terminal Q residue.
- The B1 isoform represents the unmodified isoform of Apo A1 protein, the measured mass comparing well with the calculated theoretical mass of 28078.62 amu [2].
- The C1 isoform represents either a glycosylated S, T, or N or a glycated protein isoform, the latter more likely, as plasma derived proteins are frequently glycated to some degree.
- Isoforms A2, and C2 most likely represent isoforms in which either M-84 or M-112 are oxidized and B3 an isoform where both methionines are oxidized.

References

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Acknowledgements

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