

Institution: ABRF Proteomics Research Group
Project ID: PRS-00118

I. Sample Description:

A total of two affinity purified samples containing human proteins expressed in *E. coli* were received.

1. Sample A - 5µg of protein
2. Sample B - 10 µg of protein

II. Project Goal:

To identify qualitative differences between samples A and B using isocratic ZIC[®]-HILIC fractionation followed by reversed phase LC MALDI and tandem mass spectrometry. Qualitative differences characterized include differences in protein composition and sequence coverage.

III. Experimental:

a) In-gel digestion protocol

- The two samples were dissolved in 100µL of ammonium bicarbonate in 20% acetonitrile
- The samples were then reduced and alkylated with 10µL of 250mM DTT (60min / 55°C) and 10µL of 625mM iodoacetamide (60min / room temperature / in the dark),
- Proteolytic digestion was performed in 50mM ammonium bicarbonate buffer using a trypsin to protein ratio of 1:100. The digestion was carried out overnight at 37°C.
- The digests were purified by repeated lyophilizing and reconstituting in a 0.1M acetic acid solution. After final lyophilization, the digests were reconstituted in ZIC[®]-HILIC loading buffer.

b) ZIC[®]-HILIC fractionation

- Digests of Samples A and B were reconstituted in 100µL of a 50mM formic acid solution in 90% of a 4/5 (v/v) isopropanol/acetonitrile solution.
- The samples were loaded on ZIC-HILIC trapping columns, rinsed with a 50mM formic acid solution in 95% acetonitrile and isocratically eluted with 50mM formic solution in 70% acetonitrile at a flow rate of 1.0µL/min.
- A total of 10 fractions were collected, which included 10µL of the initial rinsing solution, eight successive 5µL fractions in elution buffer and a final of collection of 20µL.
- After collection the peptide fractions were lyophilized and cleaned-up with by repeated lyophilization and reconstitution in 0.1M acetic acid solution.

c) LC-MALDI conditions

Instrument: ABI Tempo LC MALDI

Data acquisition and processing program: Tempo LC MALDI v.2.00.09

- Lyophilized digested samples were reconstituted in 20µL of 0.1% TFA in D.I water
- Injection volume - 5µL corresponding to 25% of the sample. Separation column - Merck Chromolith CapRod monolith column – 150 X 0.1mm

- Separation Gradient

Time / min	% A (0.1% acetic acid 2% acetonitrile)	% B (0.1 % acetic acid in 90% acetonitrile)
0	97	3
5	97	3
15	60	40
18	20	80
20	20	80
21	97	3
25	97	3

c) MALDI-TOF MS/MS acquisition parameters

Instrument: ABI 4800 MALDI TOF/TOF analyzer

Data acquisition and processing program: 4000 Series Explorer software

- MS acquisition in reflector mode positive ion mode
- Mass range = m/z = 850 - 4000
- 400 laser shots per spectrum
- Minimum S/N = 10 for MS acquisition
- 15 strongest precursors chosen for MS/MS
- Minimum S/N = 30 for MSMS precursors
- MALDI spot interrogated until at least 4 peaks in the MSMS spectra achieved a S/N = 70

d) LC MALDI Searching Parameters

Program for MS/MS data processing: ABI Protein ProteinPilot software 2.0

Search Engine: Paragon

Sample Type: Identification

Digestion enzyme: Trypsin

Special Factors: Gel-based I.D

Species: Homo sapiens

I.D Focus: Biological modifications

Database: uniprot_sprot_20070123+Contaminants

Confidence threshold: 66%. Lower confidence protein identifications are not reported

Results

Sample A

Proteins identified

Unused Score	Total Score	%Sequence Coverage	Accession	Name
9.24	9.24	25.74257553	Q15109 RAGE_HUMAN	Advanced glycosylation end product-specific receptor precursor (Receptor for advanced glycosylation end products) - Homo sapiens (Human)

Peptides sequenced

Contribution	Confidence %	Sequence	Modifications	Cleavages	ΔMass	Prec MW	Sc
2	99	GGDPRPTFSCSFSPGLPR	Carbamidomethyl(C)@10		-2.1784	1931.7321	11
2	99	IGEPLVLK			-0.0051	867.5378	10
2	99	IGEPLVLKCK	Carbamidomethyl@N-term; Carbamidomethyl(C)@9	missed K-C@8	-0.1239	1212.566	7
2	99	VLSPQGGGPWDSVAR			-2.1654	1522.603	16
1.05	91	VLPNGSLFLPAVGIQDEGIFR			-0.2235	2240.9922	9
0.18	34	LNTGRTEAWK		missed R-T@5	-0.1017	1174.5077	6
0.01	2	ALRTAPIQPR		missed R-T@3	-0.021	1121.6459	3

Sequence Coverage

MAAGTAVGAWVLVLSLWGAVVGAQNITAR**IGEPLVLKCK**GAPKKPPQRLQEWK**LNTGRTEAWKVLSPQGGGP**
WDSVARVLPNGSLFLPAVGIQDEGIFRCQAMNRRNGKETKSNYRVRVYQIPGKPEIVDSASELTAGVFNKVG
TCVSEGSYPAGTLSWHLDDGKPLVPNEKGVSVKEQTR**RHPETGLFTLQSELMVTPARGGDRPTFSCSFSPG**
LPRHRALRTAPIQPRVWEPVPLEEVQLVVEPEGGAVAPGGTVTLTCEVPAQPSQIHWMKDGVPLPLPPSP
VLILPEIGPQDQGTYSVATHSSHGPPQESRAVSIISIEPGEETAGSVGGSGGLGTLALALGILGGLGTAA
LLIGVILWQRRQRGEERKAPENQEEEEERAEELNQSEEPEAGESSTGGP

The color coding shows the confidence of the peptides detected for this protein

Grey – no match

Red - >0 and <50

Yellow - >=50 and <95

Green - >=95

Sample B

Proteins identified

Unused Score	Total Score	%Sequence Coverage	Accession	Name
25.6	25.6	50.2	Q15109 RAGE_HUMAN	Advanced glycosylation end product-specific receptor precursor (Receptor for advanced glycosylation end products) - Homo sapiens (Human)

Peptides sequenced

Contribution to Protein score	Confidence %	Sequence	Modifications	Cleavages	Δ Mass	Prec MW	Sc
2	99	GGDRPTFSCSFSPGLPR	Carbamidomethyl(C)@10		- 0.1579	1933.7526	10
2	99	IGEPLVLK			- 1.1111	866.4318	12
2	99	IGEPLVLKCK	Carbamidomethyl(C)@9	missed K-C@8	- 0.1165	1155.552	8
2	99	NGSLFLPAVGIQDEGIFR	Hex(1)HexNAc(1)(N)@1	cleaved P-N@N-term	- 0.1285	2297.0142	15
2	99	PGKPEIVDSASE		cleaved I-P@N-term; cleaved E-L@C-term	- 0.0273	1227.5709	9
2	99	PRPTFSCSFSPGLPR	Carbamidomethyl(C)@7	cleaved D-P@N-term	- 3.1247	1701.7158	14
2	99	TEAWKVLSPQGGGPWDSVAR		missed K-V@5	- 1.1194	2138.9507	21
2	99	VLPNGSLFLPAVGIQDEGIFR			- 0.1776	2241.0381	20
2	99	VLSPQGGGPWDSVAR			- 4.1397	1520.6288	20
2	99	VYQIPGKPEIVDSASE		cleaved E-L@C-term	- 0.0653	1730.8074	17
2	99	VYQIPGKPEIVDSASELTAGVPNK			- 0.0815	2511.2405	9
1.4	96	ALRTAPIQPR		missed R-T@3	- 0.0952	1121.5717	9
0.85	86	LNTGRTEAWK		missed R-T@5	- 0.0422	1174.5673	6
0.8	84	WGAVVGAQNITAR		cleaved L-W@N-term	- 0.1361	1341.5792	6
0.36	56	PTFSCSFSPGLPR	Carbamidomethyl@N-term; Carbamidomethyl(C)@5	cleaved R-P@N-term	- 0.1225	1508.5857	7
0.19	35	LEWKLNTGR		missed K-L@4	- 0.0983	1115.5104	6
0.01	1	PEIVDSASE		cleaved K-P@N-term; cleaved E-L@C-term	- 0.0217	945.4074	5

Sequence Coverage

MAAGTAVGAWVLVLSLWGAVVGAQNITARIGEPLVLKCKGAPKKPPQRLEWKLNTGRTEAWKVLSPQGGGP
WDSVARVLPNGSLFLPAVGIQDEGIFRCQAMNRNGKETKSNYRVRVYQIPGKPEIVDSASELTAGVPNKVG
TCVSEGSYPAGTLSWHLDGKPLVPNEKGVSVKEQTRRHPEGLFTLQSELMVTPARGGDRPTFSCSFSPG
LPRHRALRTAPIQPRVWEVPVPLEEVQLVVEPEGGAVAPGGTVTLTCEVPAQPSPQIHWMKDGVPLPLPPSP
VLILPEIGPDQGTYSVATHSSHGPPQESRAVSISIIIEPGEETAGSVGGSLGTLALALGILGGLGTAA
LLIGVILWQRRQRGEERKAPENQEEEEERAELNQSEEPEAGESSTGGP

Conclusions

1. One protein, Advanced glycosylation end product-specific receptor precursor (**accession no. Q15109**) was identified in both samples A and B with more than 99% percent confidence.
2. The sequence coverage for this protein was greater for sample B (50.2%) than sample A (25.7%).
3. A glycosylated peptide, **NGSLFLPAVGIQDEGIFR (HexNAc(1)(N)@1)**, was identified in sample B but not in sample A. A peptide, **VLPNGSLFLPAVGIQDEGIFR**, containing the glycosylated amino acid residue that was detected in sample B was also detected in sample A, but was not glycosylated.