ABRF 2008 SPRG (14005)

IN SOLUTION DIGESTION

Both samples A and B, were solved in 50 µl, 25mM Ammonium Bicarbonate, and placed in a clean Protein LoBind tube for digestion.

Trypsin digestion was performed as follows:

Reduction: incubation with 10mM DTT, 30 minutes, 56ºC.

Alkylation: addition of 55mM Iodoacetamide, 20 minutes, room temperature, dark.

Trypsin was stored at a concentration 125 ng/µl in 50mM Acetic Acid, at -80ºC. In both digestions, trypsin was used in a 1/50 ratio (protein/enzyme). At used, trypsin was diluted in an appropriate volume of 25mM Ammonium Bicarbonate.

Digestion: overnight at 37ºC.

The same procedure was performed with the original tubes, (samples A' and B'), after the addition of 30 µl, 25mM Ammonium Bicarbonate.

The liquid from tubes A; A' and B; B' were mixed and concentrated by centrifugal evaporation (SpeedVac) to a volume of ~ 60 µl.

MASS SPECTROMETRY

2/3 of the samples were analyzed by LC-MS/MS as follows:

Nanocapillary reversed-phase LC was performed with a CapLC™ (Waters Corp., Milford, MA, USA) capillary system.

Reversed-phase separation of tryptic digests were performed with an Atlantis, C_{18}, 3 μm, 75 μm × 150 mm Nano Ease™ fused silica capillary column (Waters Corp.) equilibrated in 95% ACN, 0.2% formic acid.

After injection of 40μL of sample, the column was washed with the same buffer and the peptides were eluted using a linear gradient of 5%–98% ACN for 180 min at a constant flow rate of 0.2 μL/min^{-1}.

The column was coupled online to a Q-TOF Micro (Waters Corp.) using a PicoTip nanospray ionization source (Waters Corp.). The heated capillary temperature was 80 °C and the spray voltage was 1.7–2.0 kV. MS/MS data were collected in an automated data-dependent mode. Data processing were performed with MASSLYNX 4.1 and PROTEINLYNX GLOBAL SERVER 2.3 (Waters Corp.).

PROTEIN IDENTIFICATION

Three protein identification programs were used, PROTEINLYNX GLOBAL SERVER 2.3 (PLGS2.3), PHENYX 2.3, PEAKS 4.5. PROTEINLYNX GLOBAL SERVER 2.3 and PEAKS 4.5 were loaded with raw data and PHENYX 2.3 with pkl archive obtained from PROTEINLYNX GLOBAL SERVER 2.3.

The data processing parameters used in all cases are described in the next table:

	PLGS	PHENYX	PEAKS.
Database searched (version)	Uniprot-Sprot 52.2	Uniprot-Sprot 52.2	Uniprot-Sprot 52.2
Taxonomical restrictions	No	Yes	No
Trypsin cleavage after P allowed	No	No	No
Allowed number of missed cleavages	2	2	2
Fixed aminoacid modifications	Cam	Cam	Cam
Variable aminoacid modifications	Automod	Mox	Mox
Mass tolerance	50 ppm	50 ppm	50 ppm

In all three cases only one protein has been identified in both samples, apart from our Trypsin (TRYP_PIG P00761); RAGE_HUMAN (Q15109).

There are several forms of this protein that are described in databases:

-two isoforms that are annotated in Expasy as Isoform 1 (Q15109-RAGE_HUMAN) and Isoform 2 (sp_vs-Q15109-2 RAGE_HUMAN). We only identified specific peptides from Isoform 1 in both samples.

-Q86SN1_HUMAN, Soluble form of receptor for advanced glycation end products, appears in expasy as a TrEMBL annotation. This protein has an identity of 90% with Isoform 1.

A summary of the obtained results with all search engines appears in the next table:

Sample A	**PLGS**	**PHENYX**	**PEAKS.**
Score	11.6 (max score)	69.02	99
Number of matched peptides	37	55	85
% Coverage	43	30	37
Sample B	**PLGS**	**PHENYX**	**PEAKS.**
Score	11.6 (max score)	103.31	99
Number of matched peptides	71	102	120
% Coverage	43.6	30	36

We can conclude that we have only one protein in sample A and B, and that there is no difference, in our hands, in both samples.