First Results of Proteomics Analyses in Blood Serum of Sheep in Albania

Veterinary Medicine

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Abstract

Proteomics is a comprehensive study of proteins that focuses on their structure and function. After genomics, proteomics considered the next step for the study of biological systems. But it is much more complicated than the study of genomics, because genome of an organism is more or less constant, while the proteome differs in different cells depending on weather and other factors that influence it. This science is of great interest in the fields of human and veterinary medicine. In our study we were using the method of proteomics to see the quantity and the expression of proteins in the serum of sheep in the normal (physiological) status. The tests were conducted in Mass spectrometry Laboratory–GIGA - Proteomics, University of Liege, Belgium during November 2011. After the preparation of the samples in the laboratory, a differential proteomic study using a label free LC-MSe method was conducted (nano-2D UPLC coupled to a Synapt TM HDMS TM MSe, Waters, Figure 1). The aim of this study is to create data regarding the number, name, size and the role of different proteins found in blood serum of sheep. These data will serve as reference to compare the results that will be taken from the analysis of serum to be made to the application of various proteomics study in infected animals or in study of response to immunizations by different vaccines in their prophylaxis program. In our samples we indentified 47, 45 and 49 proteins on Sheep NCBI data base in comparison with 114, 105 and 128 numbers of proteins identified respectively in each sample pool from the reviewed mammalian data base. These results need of course to be further investigated in different stress agents like: biological (infection diseases), physical and chemical agents etc.

Introduction

Proteomics is the analysis of an organism's proteome and the detection of changes in the level of Individual proteins in response to stressors such as diseases and other factors (Ambatipudi 2009; Xie 2008). Changes in serum proteins have been applied in medicine to provide information that could correlate with happen is happening in the whole organism or in an individual tissue (Villanueva 2004; Eckersall 2010; Anderson 2002). In farm animal blood, changes in the concentration of acute phase proteins can be used to assess the innate immune system's systemic response to infection, inflammation or trauma (Wait 2002; Murata 2004; Gao 2005; Ceciliani 2012).

Serum are non-invasive sample relevant for disease diagnosis because their composition Could be correlated with the overall health status of an individual animal (Talamo 2003; Soares 2012; Bendixen 2011; Apweiler 2009).
Detailed serum or plasma protein 2-DE identification maps from healthy cattle (Wait 2002), pig (Miller 2009) and sheep (Chiaradia 2012) have been described.
In the world data base we have not a full data for sheep genome sequences (Sheep database http://sheep.genomics.org.cn/home.html) and a full map of proteome for sheep (Lippolis 2008). In this context we have not data for the full analyses, names of proteins their expression etc in the blood serum of sheep.
In this work we have focused in the sample preparation of sheep serum with the aim to specifically remove high abundant proteins.These data will serve as reference for comparison with the results that will take from the analysis of serum to be made to the application of various proteomics study in infected animals (Zhong 2011) or in study of response to immunizations by different vaccines in their prophylaxis program (Mavromati 2012)

Material and methods

The study was conducted in 18 serum received from jugular vein by a flock of sheep in the Southern Albania during October 2011. Sera were transported conformity rules and were kept at a temperature of
-20ºC until initiation of analysis. Tests were conducted in Mass spectrometry Laboratory–GIGA - Proteomics, University of Liege, Belgium.
After preparation of the samples in the laboratory were conducted income forth to the following protocols:

1.Sheep serum sample preparation

We separated each sample in 6 aliquots and we created four pools with these aliquots (SFP1, SFP2, SFP3, SFP4). Only 3 first pools was analyzed in the laboratory.

2. Protein concentration determination. RC DC Kit, Protein Assay, Bio-Rad company U.S.A
We evaluated the procedures of the RC DC Kit, Protein Assay**,** Bio-Rad company U.S.A

3. 2 D-Clean Up before digestion in solution.
This kit is used to precipitate and purify the protein content from the samples. We evaluated the procedures of the 2-D Clean up kit from the company GE-healthcare USA.

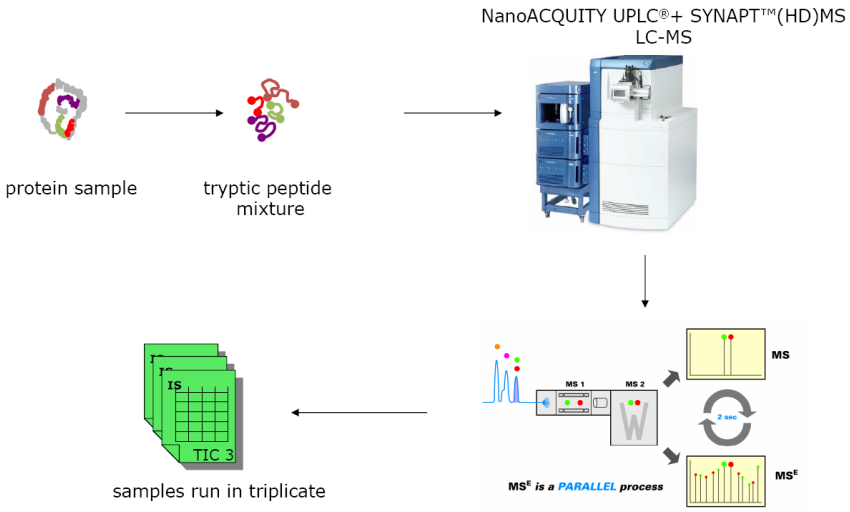
4. DIGESTIONS IN THE SOLUTION
Total digestion of the protein in solution of our sheep serum samples
4.a Reduction, 4.b Alkylation, 4.c Second reduction DTT For the neutralization of iodoacetamid.
4.d Stop the digestion with TFA (Trifluoro Acetic Acid-C2H3o2 Mw=114.02-D=1.535).

5. ZIP-TIP C18 purification
After Zip-Tip C18 purification we have in the aliquots solid peptides (dry) concentrate for the LC-MS analysis**.**

6. Preparation of the solution for the LC-MS injection.
The peptides concentrate from the samples in 3 pools (SFP 1 –SFP 3)=5 µg
Buffer formiate 200mM 9 µl(With PH =10), 100mMPDS-MIX E1 = 6 µl (Are 4 proteins : Yeast Alcohol Dehydrogenase(ADH), Rabbit Glycogen Phosphorylase b (GBP), Yeast Enolase (ENO) , Bovine Serum Albumin (BSA) in molar ratios 1.0: 0.69:0.54:0.55(+/_ 5%) The protein will be 150 fmoles in 9 µl
H20 MQ 3 µl. Vol final =18 µl. Vol of injection=9µl

7. Mass Spectra analyses. The Synapt ™ HDMS ™ G1 mass spectrometer uses electro spray ionization source (ESI) and allows sensitive detection (lower limit 25 fmole of protein) with high resolution and high mass accuracy (within 5ppm) for the analyzed peptides. This high mass accuracy is obtained by constant correction performed by co-injection of a standard peptide: (Glu)1-fibrinopeptide B (=lockmass) of known mass and known fragmentation. NanoAcquity UPLC® separation system before MS and MSE analysis of peptides uses unique combination of two C18 chromatographies performed at different pH. (This replaces the classical 2D or orthogonal separating LC steps, using successive ion exchange and C18 chromatography). All the peptides are fragmented (MS^E) and their sequence and identity can be obtained after data base searches and correlation to the accurate mass measured for each parent peptide fragmented.

Figure 1: schematic view of synapt™ HDMS™ G1 mass spectrometer



Results

1. Transfer of the data into the software.
2. Data Preparation. Software look in to the data.
3. Data identification. The Sheep NCBI database was used for the protein identification. Another database search was made against the reviewed NCBI mammalian data base.

This analysis allowed the identification of extra proteins by sequence homology between sheep and other organism. In our samples in the 3 pools, SP1, SP2, SP3 we identified respectively 47, 45 and 49 proteins.

Table nr. 1 Number of protein identification in the sheep NCBI data base and in the mammalian data base.

Categories	**Identification of the protein's number in the Sheep NCBI data base**	**Identification of the protein's number in the reviewed mammalian NCBI data base**
Sheep Pools (3 pools)		
SP1	47 proteins	114 proteins
SP2	45 proteins	105 proteins
SP3	49 proteins	128 proteins

In table nr. 1 we can see the differences in results of identification of number of proteins on Sheep NCBI data base and the reviewed mammalian data base. On the second column are the results of identification number of proteins from Sheep NCBI data base. The number of proteins is between 45 to 49 proteins in each pool. On the 3rd column are the numbers of proteins from the reviewed mammalian data base. These are between 105 to 128 proteins identified in each pool. The differences between 2 data bases are from the following reasons:

1) The sheep data base for the protein is not complete, so we have a lot of protein which are not identified yet even if the LS-MS data are available.

2) On the reviewed mammalian we have more proteins identified which are similar to sheep proteins but from other organisms.

3) The general low number of protein identification is due to the high protein concentration dynamic range in serum and the presence of high abundant proteins. In the current study no depletion or fractionation techniques were applied to the serum.

Discussions

After filtration of the data for the 3 replications of each group we have a lot of proteins, but are taxonomies for different animals, not only for sheep. This preliminary study allows us to underline some issues for the proteomic analysis of sheep sera. Basically the two major difficulties are coming from the biological matrix used.

First, serum is known to be a very complex protein mixture having in addition a variety of high abundant proteins. The number of protein identified and quantified can be drastically increase by applying depletion/fractionation or protein quantity normalization method.

Second, the sheep protein sequences database is not yet completed. To circumvent this issue we used a homology protein identification approach by searching against a Mammalian database.

Conclusions

The serum is known to be a very complex protein mixture having in addition a variety of high abundant proteins. The number of protein identified and quantified can be drastically increase by applying depletion/fractionation or protein quantity normalization method.

The sheep protein sequences database is not yet completed. To circumvent this issue we used a homology protein identification approach by searching against a Mammalian database.

These results need of course to be further investigated.

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