AMBIENT SURFACE MASS SPECTROMETRY OF MIXTURES OF SKELETAL MUSCLE PROTEINS

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Abstract – The aim of the study was to identify the skeletal muscle proteins from mixtures using Desorption Electrospray Ionizataion-Mass Spectrometry (DESI-MS) and Liquid Extraction Surface Analysis-Mass Spectrometry (LESA-MS), and further to check whether the peptides could be unambiguously identified by tandem mass spectrometry (CID MS/MS). Differentiation of all proteins from on-surface tryptic digests consisted of up to five skeletal muscle proteins, namely myglobin, troponin C, actin, tropomyosin and bovine serum albumin, was possible. *De novo* peptide sequencing was successful with the obtained data. The methods show great potential as a tool for the rapid identification of peptides from meat protein mixtures.

Key Words – Desorption Electrospray Ionization-Mass Spectrometry, Liquid Extraction Surface Analysis-Mass Spectrometry, skeletal muscle proteins

I. INTRODUCTION

Ambient mass spectrometry techniques allow the direct sampling of surfaces without any sample preparation. This approach was first introduced in 2004 with invention of desorption electrospray ionization (DESI) [1]. On-surface interactions of charged microdroplets produced from a pneumatically-assisted electrospray cause analyte desorption/ionization which results in the generation of secondary ions targeted directly to a mass spectrometer. Fully automated liquid extraction surface analysis (LESA) using a chip-based nanoelectrospray (nanoESI) system was introduced in 2010 [2]. LESA is performed by dispensing an extraction solution on the surface of the sample. After a programmed delay the solution with extracted analytes is aspirated into the tip by robotic arm and nanoESI is initiated using a nozzle of the nanoESI chip. The main advantages of ambient MS techniques are simplicity and rapidity. Therefore, the application of DESI or LESA to direct examination of proteins from complex samples, e.g. processed meat products, can results in a novel, fast method of authentication based on particular peptides.

For processed meat products, such as sausages, pâtés and ready-to-eat meat dishes, it is difficult to authenticate the animal source of the meat mainly due to the complexity, heterogeneity and considerable degradation of muscle proteins. During the last decade, many studies have been carried out in this field on the basis of proteins, DNA or stable isotopes using numerous methods, including ELISA, PCR and isotopic analysis. Merit of authenticity investigation based on peptides analysis is a possibility of application to products made from different raw materials derived from the same species. Since some proteins are tissue-specific they can be employed to authenticate processed meat products contained replacement additives, such as offal, blood plasma, mechanically recovered meat, collagen preparations, milk and egg white.

To date, a number of studies have been undertaken to show the potential of DESI for investigation of small [3] and large proteins [4], and tryptic peptides [5]. LESA has been applied successfully to the analysis of dried blood spots [6]. No one has used these techniques to analyse mixtures of skeletal muscle proteins. Previous study showed that some proteins have potential to be used as markers in authentication of meat products [7].

This work presents the results of the first part of the project supported by the EU entitled "Authentication of meat products using ambient surface mass spectrometry" (Call: FP7-PEOPLE-2011-IEF). The aim was to identify the skeletal muscle proteins from mixtures using DESI-MS and LESA-MS, and further to check whether the peptides could be unambiguously identified by tandem mass spectrometry (CID MS/MS). On-surface tryptic digests consisted of up to five skeletal muscle proteins were examined and differences between DESI and LESA techniques are discussed.

II. MATERIALS AND METHODS

Sample Preparation

Proteins, apart from BSA, were from skeletal muscles. Myoglobin (Mb, equine, 17 kDa), actin (bovine, MW 43 kDa), BSA (MW 66 kDa) and tropomyosin (TM, porcine, ~130 kDa) were purchased from Sigma-Aldrich. Troponin C (TnC, rabbit, 18 kDa) was purchased from Alpha Diagnostic Int. (USA). Proteins were dissolved in acetonitrile/H_2O (50:50) and then single proteins and their mixtures containing up to five proteins were subjected to tryptic digestion. Sample solutions were spotted onto Permanox™ slides (Nunc, Thermo Fisher Scientific, Rochester, NY, USA) at 1 µl per spot at 5 mm intervals. After evaporation of the solvent in air, 1 µl of 0.05 mM trypsin solution was spotted onto the same area. The digestion proceeded at room temperature (~24 ºC) over a period of 24 h in the humid chamber to ensure that the trypsin solution did not evaporate. After this period the solution was allowed to evaporate in air at room temp. prior to analysis.

DESI and LESA mass spectrometry

DESI source was an Omni Spray 2-D® ion source (Prosolia Inc., Indianapolis, IN). LESA source was a TriVersa NanoMate® (Advion, Ithaca, NY). Both sources were coupled to a LTQ Velos ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA) operated in positive-ion mode. The DESI operating parameters were as follows: spray tip-to-surface distance 1 mm, spray tip-to-MS inlet 3 mm, impact angle 50º, a voltage of 5 kV and 120 psi N_2 gas, spray solvent flow rate of 1.5 µl/min. The NanoMate platform operated at nanoESI voltage of 1.8 kV, gas pressure of 0.5 psi, dispensed and aspirated solvent extraction volume was 2.5 and 2.2 µl respectively. Spray/extraction solvent ACN/ H2O/FA (50:50:1) was used in all DESI and LESA experiments. Thermo raw data files were de-isotoped using the Decon software (http://omics.pnl.gov/software/DeconTools.php). Proteins were identified by Peptide Mass Fingerprinting (PMF) against the NCBI database with the assistance of the MASCOT engine.

III. RESULTS AND DISCUSSION

Detection of proteins from on-surface digests

Single proteins and their mixtures (11 variants) were examined directly from on-surface tryptic digests without any separation treatment. Fig. 1 presents LESA-MS spectrum obtained from the sampling of five-protein mixture. The mass spectra acquired in the range of *m/z* 400-1000 were very complex and noisy but many peptide peaks were readily distinguishable with good

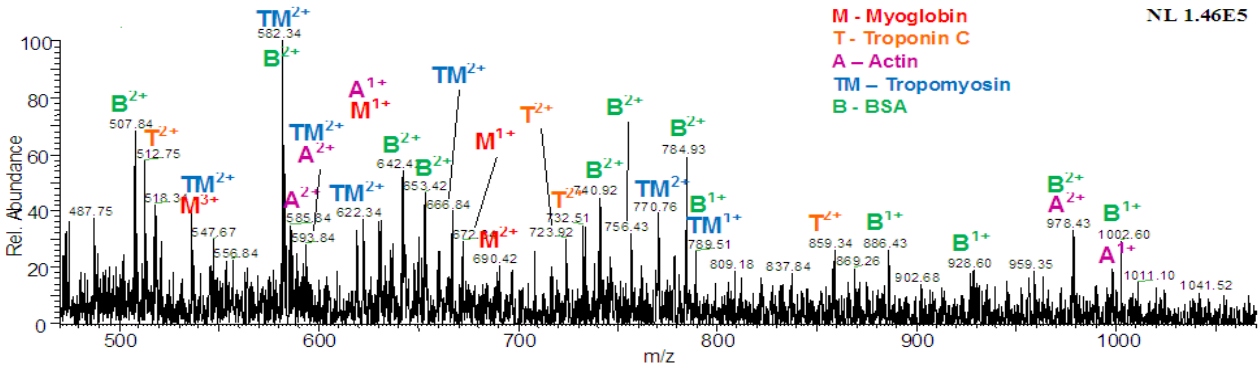

Figure 1. Positive ion mode LESA spectrum for tryptic digest of five-component mixture of myoglobin (10 µM), troponin C (55 µM), actin (24 µM), BSA (6.6 µM) and tropomyosin (0.8 µM) in ratio 1:1:1:1:1

Table 1. Muscle proteins identified from surface by DESI- and LESA-MS

Experimental protein	NCBI Accession number	Identified protein	Matched peptides[a]	Sequence coverage (%)[b]	Mascot score
Myoglobin DESI	P68082	Myoglobin (*Equus caballus*)	19	56	208
Myoglobin LESA	NP_001157488	Myoglobin (*Equus caballus*)	15	76	123
Troponin C DESI	0408496A	Troponin C (*Oryctolagus cuniculus*)	15	61	116
Troponin C LESA	0408496A	Troponin C (*Oryctolagus cuniculus*)	12	69	56
Actin DESI	NP_776650	Actin, alpha (*Bos taurus*)	26	49	198
Actin LESA	NP_776650	Actin alpha (*Bos taurus*)	21	48	172
Tropomyosin DESI	NP_001090952	Tropomyosin alpha-1 (*Sus scrofa*)	19	45	113
Tropomyosin LESA	NP_001090952	Tropomyosin alpha-1 (*Sus scrofa*)	37	83	72

[a]Number of matched peptides in the database search. [b]Percent of coverage of the entire amino acid sequence.

signal-to-noise (S/N) ratio. Differentiation of at least 3 ions derived from each protein present in the digest was readily possible.

The main differences between DESI and LESA are (a) the intensities observed in LESA were around an order-of-magnitude higher in comparison with DESI of the same samples; (b) more singly charged peaks and more peaks in the higher *m/z* region were observed using DESI; (c) more multiply charged peptides across a spectrum but reduced *m/z* range was seen in LESA. These observations are comparable with the results reported by Kaur-Atwal *et al.* [5] made on BSA tryptic peptides by use DESI/IMS and ESI/IMS. Monoisotopic peaks obtained from DESI and LESA experiments were entered manually into

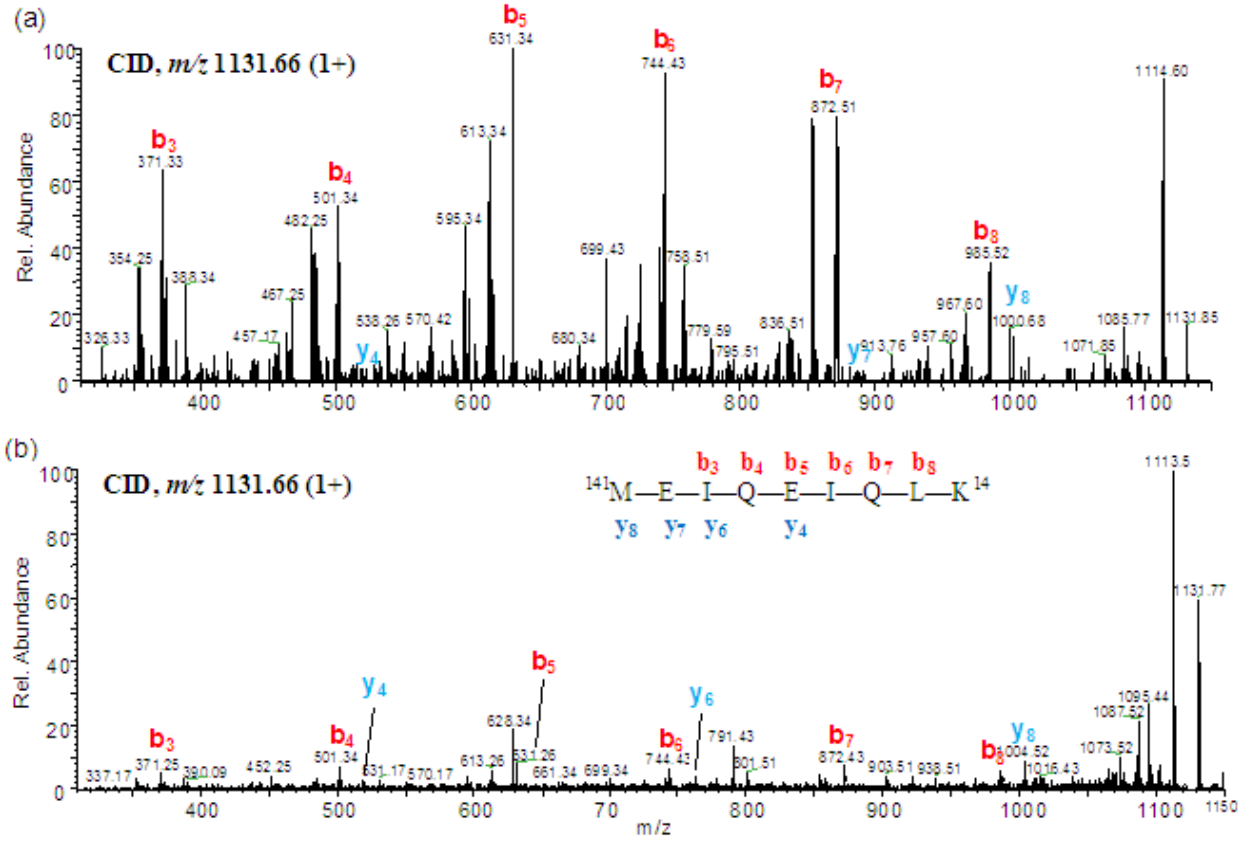

Figure 2. Average scan MS/MS spectra of the tropomyosin peptide ^{141}MEIQEIQLK^{149} (1131.66^{1+}) obtained from tryptic digest (a) DESI of porcine skeletal tropomyosin and (b) LESA of five-component mixture of myoglobin (10 µM), troponin C (55 µM), actin (24 µM), BSA (6.6 µM) and tropomyosin (0.8 µM) in ratio 1:1:1:1:1.

MASCOT for search against the NCBI protein database. Table 1 presents the search results obtained from solutions of single proteins. Proteins were identified with significant MASCOT scores of 113-208 (where > 70 was significant; $p<0.05$) and sequence coverage ≥ 45. The lower MASCOT score from LESA-generated data can likely be related to a smaller number of singly charged peptides yielded by LESA-MS. It was especially low for troponin C and tropomyosin. In the case of troponin C, the reason is that this sample was not a pure protein and some peptides characteristic for troponin T were detected, whereas tropomyosin is a large protein built of four chains Therefore, to make the calculations simpler, both manual analysis and MASCOT search were performed only across the alpha-1 chain. The difference in the MASCOT score between DESI-IMS and ESI-IMS of BSA tryptic digest has been reported previously [5]. However, data sets obtained from mixtures could not have been identified automatically through Mascot. In this case a manual processing was necessary as well as tandem MS/MS analysis.

Tandem DESI-MS/MS and LESA-MS/MS

Figure 2 shows an average scan MS/MS of the tropomyosin ion of 1131.66 *m/z* from tropomyosin digest by use DESI (a) and from a five-protein mixture using LESA (b). The spectra show the same product ions but of different intensities due to different concentration of tropomyosin in the samples. Further, the acquired spectra were used for identification through database searching. Spectra obtained from digests of single proteins were matched correctly. The top peptide matches from the data were all tropomyosin alpha-1 chain, although with human being the best much. However, peptide matched to human tropomyosin contained the same sequence as porcine tropomyosin. The MASCOT match of spectra acquired from protein mixtures turned out to be incorrect. In this case MASCOT engine was not suitable for confirmation of the identity.

IV. CONCLUSION

The results demonstrate the suitability of direct surface sampling of protein mixtures by use of DESI and LESA mass spectrometry for the unambiguous identification of skeletal muscle proteins and peptides. No separation step is required. The entire analysis including the following MS/MS takes ~10 min (excluding sample preparation). The methods show great potential as a tool for the rapid identification of peptides from meat and complex meat products.

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