Proteomic identification of the bovine pregnancy associated proteins by peptide mass fingerprinting

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ABSTRACT

In this study, a method for the localization of pregnancy-specific proteins from cow urine on 2-D gel have been established. The proteins were digested with trypsin in gel and then analyzed with MALDI-TOF or transferred to a membrane and microsequenced. To examine the pregnancy associated protein spot 2 as a diagnosis marker in bovine urine 2-D western blotting was performed. This antibody was reacted specifically in the protein of pregnant cow's urine. Consequently spot 2 was identified and found to be good candidates for developing cow pregnancy detection assay kit.

INTRODUCTION

The proteome map of normal urine of Holstein, the most common species of dairy cow in Korea and all over the world, was made at first, and then to examine the application the map of pregnant cow's urine was made to find pregnancy-associated protein using as a marker of cow pregnancy diagnosis. This proteome map to characterize the protein is showing differences between the two conditions MALDI-TOF Mass spectrometer and Q-Tof MS/MS.

MATERIALS AND METHODS

In-gel digestion of the protein: The protein spots of interest were excised from the gel, minced with scalpel, and placed in a 0.5 ml siliconized Eppendorf centrifuge tube. With 400㎕ 50% acetonitrile in 25 mM ammonium bicarbonate, the gel was washed three times. The pieces were dehydrated with 100 ㎕ of acetonitrile and the gel dried in a vacuum centrifuge. For trypsin in-gel digestion, 5 ㎕ digestion buffer (10 ng/㎕ trypsin in 25 mM ammonium bicarbonate) was added, and after 10 minutes the additional digestion buffer without trypsin was added to submerge the gel slices. The mixture was incubated for 24 hours at 37℃, after completion of the digestion, the supernatant was transferred into a new tube. Following enzymatic digestion, the resultant peptides were extracted three time with 30 - 50㎕ of 0.1% trifluoroacetic acid in 50% acetonitrile, and

dried completely by vacuum centrifugation. The digests were re-dissolved in 0.1% TFA, and were purified with ZipTip c18 pipette tips (Millipore, Bedford, MA, USA), using the procedure recommended by the manufacturer. The peptides were eluted from the tip directly onto the MALDI plate with a solution of CHCA (10mg/ml in 50% acetonitrile/ 0.1% TFA).

Matrix-assisted laser desorption ionization time-of-flight (MALDL-TOF) MS: The measurements were performed on a Voyager DE-PRO MALDI-TOF mass spectrometry (Applied Biosystems, Framingham, MA, USA). The instrument is equipped with a nitrogen laser (λ=337 nm, 3 ns pulse width), a delayed extraction (DE) source, a gas cell for collision-induced dissociation (CID), and a single-stage reflector (R). A Tektronix TDS 540B digitizing oscilloscope (Tektronix, Inc., Wilsonville, OR, USA), with 500 MHz analog bandwidth and a 2 gigasamples/s mix. digitizing rate, was used for data acquisition. The MALDI spectra were acquired in the delayed extraction, reflector mode, using standard conditions (20kV acceleration voltage, 150 ns delay time). The mass scale was calibrated internally with the trypsin auto digestion products of known amino acid sequence, $[M+H]^+$ = 515.33 (fragment 54-57) and 842.51 (fragment 108-115). Typically, 100 scans were averaged to produce the final spectrum. Based on the database search results, 69% of the measured tryptic peptide masses matched within 50 ppm to the corresponding theoretical masses; 88% of the measured masses matched within 100 ppm.

BLAST protein search program of NCBI: The BLAST (Basic Local Alignment Search Tool) programs introduced a number of refinements to database searching that improved overall search speed and put database searching on a firm statistical foundation (Altschul et al., 1990) A region of the query sequences can be used to be used for BLAST searching. The range in nucleotides or protein residues in the "From" and "To" boxes provided under "Set Subsequence" can be entered. For example to limit matches to the region from nucleotide 24 to nucleotide 200 of a query sequence, "From= 24, To= 200" would be enterd. If one of the limits entered is out of range, the intersection of the [From, To] and [1, length] intervals will be searched, where length is the length of the whole query sequence. The BLAST pages offer several different databases for searching. Some of these, like SWISS-PROT, PDB and Kabat are complied outside of NCBI. Other like ecoli, dbEST and month, are subsets of the NCBI databases. Other "virtual Databases" can be created using the limit by Entrez query option.

RESULTS AND DISCUSSIONS

The proteins in the fractions were separated on the gels and compared in terms of expression level and new expression at the same molecular weight and isoelectric point

with an image analysis program, PDQuest(Fig. 1, Table 1). Some common and pregnancy-associated protein spots were excised and digested with trypsin, and then analyzed with MALDI-TOF. With this peptide mass fingerprint, the proteome map of bovine urine was made(Fig. 1). To rule out the possibility that environmental or genetic factors might influence the expression of the proteins, we demonstrated the pregnancy-associated expression of the proteins in two-dimensional gels with the pregnant urine taken from the cows raised in a different institute. One of the pregnancy-associated proteins with molecular mass of 20 kDa, namely spot 2, was microsequenced and found to be highly homologous to bovine collagen alpha 1 chain(Table 2).

REFERENCES

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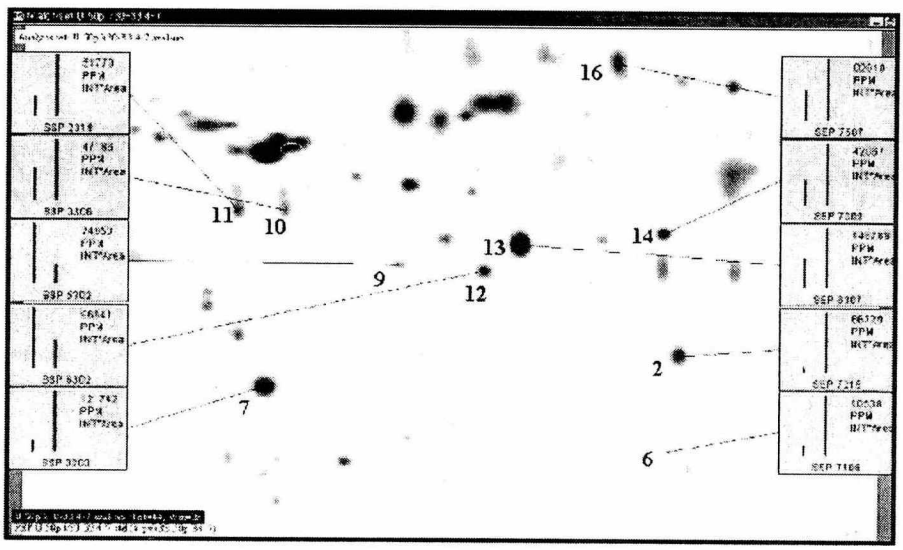

Fig. 1. Analysis set using 2-D image analysis program. The differences between pregnant and non-pregnant cow's urine protein was showed in the margin graphs. As the spot intensity has differences over two folds each other, the graph was showed in the margin. The left bar in margin graph indicates non-pregnant and the right bar indicates pregnant.

Table 1. Catalogue of urine proteins for MALDI-TOF MS analysis verified by a PDQuest image analysis program

Spot Number	Molecular Weight (Da)	pI	Protein expression during pregnancy
2	20,000	5.7	increased
5	18,000	5.7	increased
6	19,000	4.8	increased
7	22,000	4.3	constant
8	24,000	5.4	decreased
9	28,000	4.9	increased
10	28,000	4.7	increased
11	24,000	5.5	decreased
12	26,000	5.6	increased
13	27,000	5.7	increased
14	33,000	5.6	constant
15	36,000	5.7	increased
16	38,000	5.6	increased
17	38,000	5.5	increased

Table 2. Matched parts of peptide sequences with top ranked protein of the spot 2 using MS-Fit searching program

m/z submitted	MH+ matched	Delta Da	Start	End	Peptide Sequence	Modifications*
933.3974	933.4390	44.5631	635	643	(K)GSQGEKGDR(G)	
2451.2695	2451.2818	5.0334	463	488	(R)TLALMGPPGLPGQTGPPGPPG TPGQR(G)	
2467.2565	2467.2768	8.2086	463	488	(R)TLALMGPPGLPGQTGPPGPPG TPGQR(G)	1Met-ox
2484.2989	2484.2054	37.6463	683	709	(K)GDPGMTGPTGAAGLPGLHGP PGDKGNR(G)	

* Met-ox indicates an oxidized methionine, and peptide mass tolerance (+/-), was 50ppm.