DIFFERENTIALLY EXPRESSED PROTEINS IN FOUR GROWING STAGES IN CHICKEN LIVER AND MUSCLE

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Key Words: Chicken, liver, muscle, growing stages, 2DE, MALDI-TOF MS

Introduction

Proteomics is a recently developed technique and giving information of all profiles of proteins in a specific tissue or cell. Even though different cells have the same set of genes, the expression pattern of the proteomes differs for different tissues or cells. Therefore, proteomic study includes protein-protein interaction in a given environment and the identification of expressed and modified proteins (Pandey and Mann, 2000). Among the animal species, chicken has been contributed to understanding of regulation of skeletogenesis, myogenesis, embryonic patterning and limb development. Also, the chicken offers to understanding of lipoprotein metabolism and fattening (Hermier, 1997). In this study, chicken liver and muscle proteomes have been investigated in order to understand basic metabolisms, mainly identifying major proteins for lipogenesis and muscle development.

Materials and Methods

Liver and skeletal muscle samples were collected at 0, 10, 21 and 32 weeks of age from White Leghorn and Korea native chicken, respectively. Approximately 200 mg of each sample were used for protein extraction.
For first dimensional separation, 1 mg protein of each sample was loaded onto immobilized pH-gradient (IPG) strips (pH 3-10NL and pH 3-10L, 18 cm: Amersham) and rehydrated for 12 hr. Focusing was started at 100 V and gradually in 1000 V for 1 hr increased to a final voltage of 8000 V at 20℃. After equilibration, second dimension was run on 8-16% linear gradient SDS-polyacrylamide gels. Staining was carried out according to the method of Heukeshoven and Dernick (1985) using a Coomassie Brilliant Blue G-250 (Fluka, Germany). After in-gel trypsin digestion, peptides of liver samples were analyzed using a modified Voyager-DE STR MALDI-TOF mass spectrometer (PerSeptive Biosystems, USA). The proteins were identified by searching using MASCOT peptide mass fingerprint software and profound program. The identified interesting proteins were further investigated using western blotting.

Results and Discussion

Differentially expressed proteins were detected from liver and muscle of White Leghorn and Korean native chicken at four different growing stages. Based on 2-DE results, approximately 700 protein spots from liver and muscle samples were detected using coomassie staining. Most of the proteins were appeared in the pH 5-9 range and molecular weight 20-100 kDa (data not shown). Generally, similar muscle protein patterns were obtained compared with the results of Doherty et al. (2004). Previously, no one investigate the chicken liver protein patterns and this is the first results of 2DE patterns of chicken liver. Currently, the differentially expressed chicken muscle proteins in four growing stages are being investigated and the results are not included in this paper. However, the differentially expressed chicken liver proteins in four growing stages were fully investigated. Since the chicken liver is the key organ for fat metabolism and very important for the fat related economic traits.

Based on the comparison of four different growing stages, thirteen differentially expressed protein spots were selected in chicken liver. These proteins were further analyzed by MALDI-TOF MS and then were searched against database search using MASCOT peptide mass fingerprint software and profound program. Detected proteins were listed in Table 1. Twelve protein spots were identified as known proteins and one spot was unknown protein based on the search against Swiss-Prot and the National Center for Biotechnology Information non redundant (NCBInr) database.

The proteins fatty acid synthase (FAS, No. 6), malate dehydrogenase (MDH, No. 8) and malic enzyme (ME, No. 9) which are differentially expressed with growth stages are specially related to lipid metabolism. The expression comparison indicated that the FAS was continually over expressed in 0, 10, 21 and 32 weeks with growth. Especially, the difference in expression levels of the spot 6 between 0 week and 32 weeks was around 3 times. For the growth stages of 0 to 32 weeks, the MDH, ME was not lineally expressed differentially on the growth stages of 0 to 21 weeks. But the MDH was more expressed roughly 3 times comparing on the 31 than 0 stages and ME was more expressed roughly 7 times comparing on the 31 than 0 stages. To confirm this result, FAS of truly up-regulated pattern was validated by western blotting with antibodies against FAS protein. The consistent result was obtained using the western blotting data as compared with previous 2-DE results (Figure 1).

Table 1. The identification results for the differentially expressed proteins in four growing stages in chicken liver.

Spot ID	Top Score[a], Est'd Z[b]	Accession No	Protein Information	%	pI	kDa
1	-	-	-	-	-	-
2	156	gi|45383974	albumin [Gallus gallus]	39	5.51	69.872
3	157	gi|50752703	similar to Sorbitol dehydrogenase (L-iditol 2-dehydrogenase) [Gallus gallus]	52	7.09	38.12
4	147	gi|62738642	Chain A, Crystal Structures Of Chicken Annexin V In Complex With Zn2+ [Gallus gallus]	39	5.61	36.045
5	2.34	XP_421496.1	similar to catalase [Gallus gallus]	31	7.3	55.77
6	120	gi|1345958	Fatty acid synthase [Includes: [Acyl-carrier-protein] S-acetyltransferase [Gallus gallus]	7	5.94	274.607
7	2.18	XP_421496.1	similar to catalase [Gallus gallus]	30	7.3	55.77
8	143	gi|57530355	malate dehydrogenase 1, NAD (soluble) [Gallus gallus]	34	6.92	36.52
9	156	gi|15420977	malic enzyme [Gallus gallus]	30	6.45	61.961
10	89	gi|57524986	heat shock 70kDa protein 9B (mortalin-2) [Gallus gallus]	17	6.09	73.141
11	2.39	XP_421496.1	similar to catalase [Gallus gallus]	26	7.3	55..77
12	131	gi|230360	Chain B, Structure of Triose Phosphate Isomerase from Chicken Muscle [Gallus gallus]	38	7.26	26.527
13	85	gi|46048768	enolase [Gallus gallus]	24	6.17	47.275

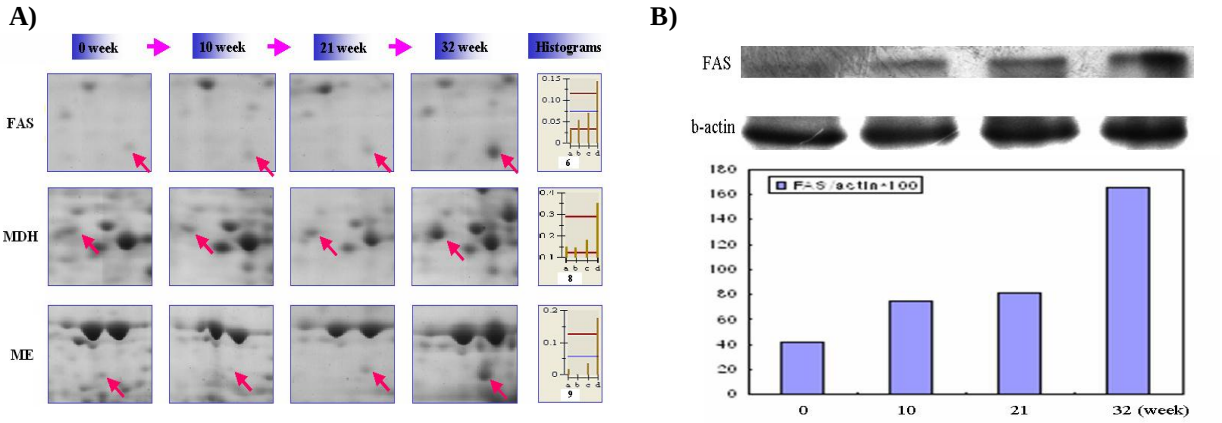

Figure 1. Differentially expressed proteins in relation to lipid metabolism in four growing stages in chicken liver (left). Western blot analysis of FAS protein expressed in chicken liver (right).

Conclusions

The differentially expressed proteins in chicken liver and muscle for four growing stages (0, 10, 21, 32 and 0, 10, 21, 35) weeks of age were investigated. Thirteen growing stage specific proteins in liver were selected and characterized by MALDI-TOF MS. Currently the differentially expressed proteins in muscles have been being investigated. Three lipid-related differentially expressed proteins were identified in chicken liver. Upon finishing, these results will give some useful information for understanding lipid metabolism and muscle development in chicken.

References

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