A PROTEOMIC APPROACH TO COMPARE MEAT FROM DIFFERENT SPECIES

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Background

Proteome analysis has become an essential part of the developing field of "functional genomics" and will be evolved into a high- throughput technology for the study of global protein regulation. Actually, proteomics is much older than genomics and in principles dates back to the mid 70's with the independent introduction of the two-dimensional gel electrophoresis technique (2DE) by lose and O' Farrel. This allows separation of cellular proteins according to molecular mass and isoelectric point. In the beginning, around 300 protein spots from mouse tissue could be separated. Now, with the refined technique developed by Klose's group, maximum resolution has been achieved which allows of more than 10000 protein spots under reproducible condition. This has the potential to reveal almost all proteins of a specific cell type, its proteome. The term "proteome", however, was coined not until 1994, as an equivalent to genome. Already in first paper, Klose considered not only qualitative but also quantitative differences in the amount of individual proteins. In the late 70's, the first protein databases were established which marked the beginning of the large scale study of cellular proteins, i.e. proteomics.

Objectives

The aim of this work is to compare, with the aid of two dimensional electrophoresis and proteomics, meat from differences species. There are no databases about proteome of meat; in 1998 we previously report a refer work of comparison between difference fish fillets. The database on web and the published two dimensional maps report only muscular tissue of human and mouse.
We analysed four types of meat, bovine, swine, rabbit and chicken. The results are very interesting because there are evidence of conservation of housekeeping proteins interspecies and there are lots of differences between glycolysis and Krebs cycle proteins.

Methods

Pretratment of samples for 2D pages involved solubilization, denaturation and reduction to completely break up the interaction between the proteins. 0,1 g of meat tissues is suspended in 1 ml of lysis buffer, then are homegenized in a cooled mortar, centrifuged and filtered. The composition of lysis buffer is 9.5 M urea, 25% w/v CHAPS, 2% Pharmalite pH 3-10, 1% DTT and 5 mM Pefabloc . The final protein concentration lies 5 mg/ml. Isoelectric focusing was performed using immobilized pH gradient strips (home made), which had a linear pH range of 3-10 (18 cm long). The samples were applied to the strips using the in-gel rehydration method, as described by Rabilloud et al. and Sanchez et al. The samples were diluted to a total volume of 350 µL in rehydration solution, which contained 8M urea, 0.5% CHAPS, 15mM DTT, 0.2% Ampholine (3-10, Amersham Biosciences). The strips were rehydrated and focused on an IPGphor (Amersham Biosciences) at 20°C. Rehydratation was allowed for 10 hours under low voltage (5 hours at 30V and 5 h at 50V). Focusing obtained by application of 500 V for 1 h, 1000 for 1h, 8000 for 10 h. The current was limited to 50 mA per strip. The equilibration step was performed for 22 min. The equilibration solution was 6M urea, 2%SDS, 30% glycerol in Tris-HCl buffer, pH 6.8, plus DTT for 15 min that was replaced by IAA. Second dimension was carried out on to Excel Gel SDS, homogenous, 12.5% (Amersham Biosciences), the run was performed for about 2 hours, at 600V, 30mA, 50W. Matching was performed with Image Master 2D Elite, a dedicated software for compare two dimensional maps, and the recognize of proteins was made on line, **http://www.expasy.ch/cgi-bin/map2/def?MUSCLE_MOUSE.**

Results and discussion

By comparison of silver stained derived from different animal species two different groups of proteins were identified:1. housekeeping proteins, like actin, myosin, tropomyiosin and albumin, with acidic pI and molecular weight from 20000 to 60000 Da; the relative position on to 2D map are the same for the four species, and the albumin is more expressed in swine tissue (probably due to his able to link fatty acids); 2. creatin kinase and his isoforms (pI 6-7, MW 60000) and 3. glycolysis and kreb's cycle zone, more accentuated in rabbit muscle and chicken. This technique may be an election method to identified sophistications in meat mixture or the degree of tenderisation; our scope, due to the poor literature about these problem, is to build a meat index proteins, with help of mass spectrometry, the fundamental complement of proteomic science.

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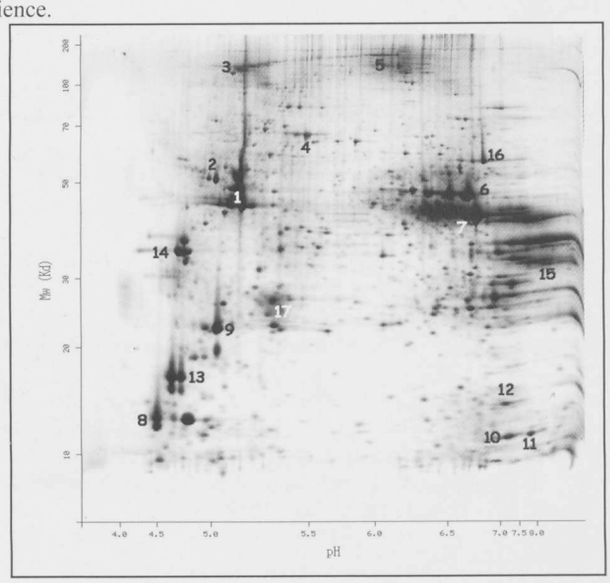
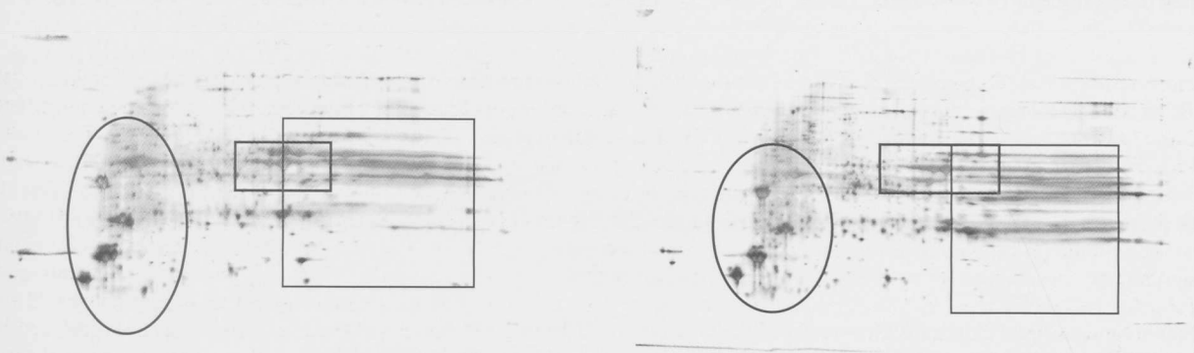


Figure 1. Electrophoretogram of muscle mouse, **http://www.expasy.ch/cgi-bin/map2/def?MUSCLE_MOUSE**

1.actin 2. ATP synthase 3.myosin heavy chain 4 serum albumin 5. skelemin 6. beta enolase 7. creatin kinase 8 myosin, light chain 9, idem 10, emoglobin, beta chain 11 emoglobin alpha chain 12 myoglobin 13myosin light chain, regulatory protein 14 tropomyosin 15 porin (VOC channel) pyruvate kinase 17 endopeptidase

Figure 2 electrophoretograms of bovine muscle (left) and rabbit muscle (right) , swine muscle (left down) and chicken tissue (right down). Ellipse: housekeeping proteins zone; rectangle: creatine kinase isoforms zone; square: glycolysis and Krebs cicle zone



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