

Proteomics and some of its Mass Spectrometric Applications

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Introduction

Proteomics is screening of proteins, protein expression, protein modifications and protein-protein interactions. In high-throughput analysis approach several proteins are analyzed at same time.

Currently human genome and many other species genomes has been sequenced and genes counted and identified (~25000--30000 in human), but number of genes is not equal to number proteins. Swiss-Prot protein database (release 46.4, 29th of March 2005) has over 178000 protein sequence entries, therefore research on protein functions is really important.

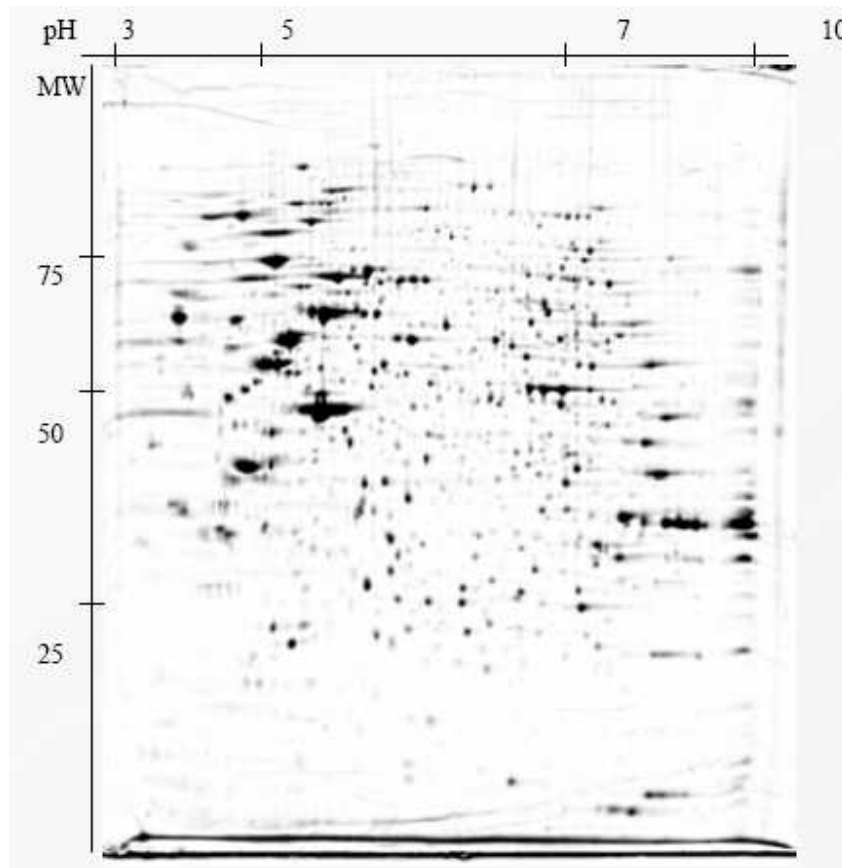
Proteomics

Proteomics can be divided to several subgroups

- Proteomic analysis (analytical protein chemistry) is characterization of proteins (amino acid sequence) and post-translational modifications (i.e forming of disulfide bridges, etc.).
- Expression proteomics is expression level profiling using quantitative methods and comparison of these expression levels.
- Cell-mapping proteomics is analysis of protein-protein-interactions (how proteins work together).

Gel-based proteomics

Gel-based proteomics was developed 1970's. Proteins are separated in Two-Dimensional gel Electrophoresis (2DE). First dimension separation is based on Iso-Electric point (pI) of protein, which is pH, second dimension is based on molecular weight of protein. In picture 1, x-axis is the Iso-Electric point and y-axis is the molecular weight (MW) and dark dots are proteins.



Picture 1.

In quantitative proteomics gel images of cases and controls are compared. 2DE method can be used analyzing hundreds (~800-1500) of proteins at the same time and possible post-translational modifications can be identified. Method is time-consuming and requires quite lot of manual laboratory work.

Protein arrays

Idea of protein arrays is similar as cDNA arrays. Technology is ~10 years old. Substrate is bound on the surface of the array. They are detected and analyzed. Protein arrays are used for screening, profiling, interactions, etc. Problems with

this method is that antibodies are expensive, some proteins bind to everywhere and background variation.

Yeast two-hybrid system

Traditional way to analyze protein-protein interactions, where it is very successful, which was first used with yeast (*S. cerevisiae*), but it has been applied to other organism too. Problems with this method is example that if post-translational modifications are required for binding, interactions are difficult to detect or some proteins are never expressed at the same time.

Mass Spectrometric techniques

Mass spectrometric (MS) are performed after quantitative analysis by Two-Dimensional gel Electrophoresis (2DE). Techniques are peptide mass fingerprinting (Maldi MS), sequence based identification (MS/MS). Proteins are digested different enzymes. Then peptide mass is measured with TOF-mass analyzer, where “fly-time” of protein is measured. Then example MASCOT peptide mass fingerprinting database can be used for later analysis.

ICAT

ICAT method is quite new and it was developed 1999. Basic idea of ICAT method is that only cystein is labeled. Then MS/MS is used. Problems with this method is that analysis software is not really good yet.

Conclusions

Rapid development of new detection machines and methods have not eliminated all laborious work. Current methods are good for case-control studies.

References

Strachan, T. and Read, A.P., Human Molecular Genetics, Third Edition. BIOS Scientific Publishers Ltd, 2003.

Swiss-Prot: Protein sequence database. <http://au.expasy.org/sprot/>

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