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The Analysis of Proteomics on the basis of Mass Spectrometry and 2-Dimensional Electrophoresis

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Abstract:

Mass spectrometry (MS) is a widely recognized as a powerful tool for analyzing biological and molecular samples that is involved into an indispensable tool for proteomics research. By using mass spectrometry, identification of Two-dimensional gel electrophoresis (2-DE) with immobilized pH gradients (IPGs) that is combined with protein and workhorse for proteomics. Mass spectrometry is used by researcher for identify, quantify and characterize biomolecules like proteins from any number of biological conditions or sample types. 2-DE currently technique that can be applied for parallel quantitative expression profiling of large sets of complex protein mixtures such as whole cell lysates. Separation of complex mixture of proteins according to isoelectric point, molecular mass, solubility and relative abundance by using 2-DE. In this paper, discuss about the mass spectrometry for proteomics and 2-dimensional electrophoresis in proteomics.

Keywords: *Mass Spectrometry (MS), 2-dimensional Electrophoresis, Isoelectric point, Solubility*

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Introduction

Proteomics is defined as the protein complement of the genome and involves the complete analysis of all protein in given sample. It contain several technologies such as mass spectrometry, 2-Dimensional Electrophoresis (2-DE), bottom-up, Liquid Chromatography etc. In this paper, we will only discuss about the mass spectrometry and 2-DE.

2-dimensional Electrophoresis in Proteomics

In 2D gel-based proteomics, 2-D gel part represent the essential workload of whole process. Quantitative analysis is performed at this step and this quantitative analysis is usually used to perform spot selection. It contain important significances for the downstream mass spectrometry analysis. First, only need to be small sample of protein that is present in the sample and will need to be analyzed. If we imagine ten samples to be analyzed and compared, per sample take 20 hours of mass spectrometry that represent in shotgun-type techniques 200 hours of MS. This sample analysis is carried out with 2D gels, 20 different spot will be selected at the end of image analysis and represent at very most 20 hours of MS. It does not mean that 2D gel-based proteomics is more productive per se but it means that the burden put into more expensive and reduced the MS part in this scheme. When analyzing the comparative productivity of 2D gel-based proteomics and shotgun type proteomics, it appears when the size of the sample series increases, then compared productivity of 2D gels over shotgun improved due to the highly parallel nature of 2D gel-based proteomics.

Second, MS is very simple and cheap that can be used to identity a protein from a 2D gel due to the high resolution of 2D gels. For example, old peptide mass fingerprinting method is fairly cheap, fast and can be carried out on low-price TOF MS that is works only with 2D gel-separated proteins and it does not work with any other technique of less resolving power.

There are also some niche applications where special traits of 2D gel-based proteomics are taken to profit. One example is micro enzymology where 2D electrophoresis is used as a protein micropreparative tool. Another example is immunoproteomics, where it is immune response of patients that is proved at a proteomic level. In this case, using these:

- 2D gels are interface with antibody probing through the classical blotting process.
- The fact that the resolution of 2D gels is such that in the identification stage, there is very little chance that the identified protein does not correspond to the immune detected one, while the probability of co-migration would be much higher in less resolutive systems.

When post-translational modification re studied, then 2D gels are also very appropriate. First, same procedure of blotting is follows with antibodies directed against a modification such as tyrosine nitration and citrullination. Second, many post-translation modification do alter the pI and or the MW of proteins and induce position shifts in 2D gels such as glycosylation and phosphorylation.

Mass spectrometry

Protein can be investigated on a large scale by using toolkit technique in which mass spectrometry (MS) are most popular because of its ability to handle the intricacies associated with the proteome. Mass spectrometry of proteomics have three applications such as protein expression, defining protein interactions and identifying sites of protein modification. The use of mass spectrometry for proteomics is not application of a single technique for all purposes but relatively a collection of methodologies. Consideration should be given to the type of instrumentation and fragmentation in MS experiment to an individual sample.

Mass spectrometric instrumentation for proteomics

Basically, gas-phase ions are measured by mass-to-charge ratio (m/z) in MS. Mass spectrometers consist of an ion source that convert analyte molecules into gas-phase ions, separates the ionized analytes that based on mass-to-charge ratio by mass analyzer (central of MS technology) and detector that records the number of ions at each m/z value. Mass analyzer contain the four types that is commonly used in proteomics: quadrupole (Q), ion trap (quadrupole ion trap, QIT; linear ion trap, LIT or LTQ), time-of-flight (TOF) mass analyzer, and Fourier-transform ion cyclotron resonance (FTICR) mass analyzer. These mass analyzer contain the many characteristics that is given in below table.

Table: Characteristics of commonly used in Mass analyzer in Proteomics

Instrument	Mass Resolution	Mass Accuracy	Mass-to-charge Range	Ion Source	Main Application
Quadrupole ion trap (QIT)	1000[a]	100-1000ppm	50–2000; 200–4000	Electrospray Ionization (ESI)	Protein identification of low complex samples; post-translational modifications (PTM) identification
Linear ion trap (LIT)	2000[a]	100–500 ppm	50–2000; 200–4000	Electrospray Ionization (ESI)	High throughput large scale protein identification from complex peptide mixtures by on-line Liquid chromatography mass spectrometry (LC-MS^n); post-translational modifications (PTM) identification
Q-q-Q	1000	100–1000 ppm	10–4000	Electrospray Ionization (ESI)	Quantification in selected reaction monitoring (SRM) mode; PTM detection in precursor ion and neutral loss scanning modes
Q-q-LIT	2000[a]	100-500ppm	5-2800	Electrospray Ionization (ESI)	Quantification in selected reaction monitoring (SRM) mode; PTM detection in precursor ion and neutral loss scanning modes
Time-of-flight (TOF)	10,000–20,000	10–20 ppm[b]; <5 ppm[c]	No upper limit	Matrix-assisted laser desorption/ionization (MALDI)	Protein identification from in-gel digestion of gel separated protein band by peptide mass fingerprinting
TOF-TOF	10,000–20,000	10–20 ppm[b]; <5 ppm[c]	No upper limit	Matrix-assisted laser desorption/ionization (MALDI)	Protein identification from in-gel digestion of gel separated protein band by peptide mass fingerprinting or sequence tagging via Collision induced dissociation (CID) MS/MS.
Q-q-TOF	10,000–20,000	10–20 ppm[b]; <5 ppm[c]	No upper limit	Matrix-assisted laser desorption/ionization (MALDI)	Protein identification from complex peptide mixtures; intact protein analysis; PTM identification
Fourier-transform ion cyclotron resonance (FTICR)	50,000–750,000	<2 ppm	50–2000; 200–4000	ESI and MALDI	Top-down proteomics; high mass accuracy PTM characterization
LTQ-Orbitrap	30,000–100,000	<5 ppm	50–2000; 200–4000	ESI and MALDI	Top-down proteomics; high mass accuracy PTM characterization; protein identification from complex peptide mixtures; quantification

Fragmentation method

Tandem mass spectrometry is a key technique that is used for protein or peptide sequencing and post-translational modification analysis. It is introduced as an additional level to mass fingerprinting. It contain many ways for broken of peptides into smaller molecules (fragments). Peptide fragmentation can be induce into many ways such as by collision with an inert gas. When peptide fragmentation is done then created the two molecules: prefix and suffix. Fragmentation process can occur on multiple copies of peptides in which many prefix and suffix are observed. In this method, gas-phase peptide/protein cations are internally heated by multiple collisions with rare gas atoms. This leads to peptide backbone fragmentation of the C–N bond resulting in a series of b- and y-fragment ions. Energetic feature associated with this method due to the slow-heating and internal fragmentation and neutral losses of H2O, NH3, and labile PTMs are common. It give results in limited sequence information for large peptides (>15 amino acids) and intact proteins.

Review of Literature

Campbell 2003, in this paper, requirement of teacher for research and teaching on genomics. After hiring of teacher, teacher improve their teaching and research simultaneously, then next generation of faculty will believe that teaching and research are mutually beneficial rather than mutually exclusive.

Görg, Weiss and Dunn 2004, concluded that diversity of emerging the proteomics platforms in which there is no another applicable method that replace the 2-DE in its ability to simultaneously separate and display thousand proteins from complex samples such as microorganisms, cells and tissues. It is used Immobilized pH gradients (IPG) that proved to be flexibility with respect to requirements of proteome analysis. IPG deal with MS that for separating and identifying complex protein mixtures in proteomics projects.

Encarnación et al. 2005, concluded that at functional molecular level, proteome has recently emerged that should help to loosen biochemical and physiological mechanisms. By using two dimensional polyacrylamide gel electrophoresis, proteome analysis methodologies separate proteins that is followed by powerful peptide mapping approached in which gel-embedded proteins are enzymatically sliced, peptide products are eluted, and identity determined by database searching of the masses or sequences derived from MS analysis of those products.

Lamont et al. 2006, dictated that demand for high throughput protein expression applications at the level of measuring actual protein will only continue to rise. This paper goal of globally describing the response of P. gingivalis to its human host during the various stages of invasion and internalization requires a complete dataset that including transcription measurements for each gene, quantitative measurements and differential measurement of modification status of each protein. Proteomics will likely be drive for future development that include both analytical method and software.

Colinge and Bennett 2007, concluded that proteomics play an important and ever-increasing role in biological research and generate large quantities of data by available technologies. Bioinformatics contain the open challenges for analysis of data that require modelling and processing. It contain best example that is mass spectrum which is continuous and discrete information simultaneously.

Kumar et al. 2017, stated that 2D electrophoresis techniques are used for proteome that is identification of biomarkers, cancer research, and cell differentiation and so on. To improve the resolution of erythrocyte membrane proteins in various conditions in 2-DE. Quality of 2-DE analysis primarily depends on the quality of protein sample preparation that is observed in this study. Improved the solubilization of proteins by increasing the concentration of zwitter ionic detergent 1-propanesulfonate (CHAPS).

Conclusion

Proteomics tool play an important role for the interrogation of protein expression, protein interaction and protein modification by the development of new instrumentation and fragmentation methods. Mass spectrometry incorporated into modern day biology that is contributed important insights into complex biological processes such as embryonic stem cell biology, kinase signaling network and disease. Qualitative and quantitative complete characteristics of proteome that is a tremendous challenge. 2D electrophoresis is mature technique that offers great flexibility and tunable resolution and available many tricks to refine the resolution. No other method is applicable which replace the 2D electrophoresis method in its ability to simultaneously separate and display several thousand proteins from samples such as microorganisms, cell and tissues.

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