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A REVIEW ON PROTEOMICS AND THEIR TECHNIQUES

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ABSTRACT

Proteomics can be defined as the functional analysis of a complete set of proteins using high throughput technologies in a system. The proteomics workflow is a multi-step process involving sample preparation, separation, quantification and identification of proteins. Due to the high complexity of the various types of proteins and the wide dynamic range of the amount of proteins in the cell system, it is necessary to apply appropriate analytical methods. Proteomics involves the use of technologies to identify and quantify the total content of proteins present in a cell, tissue, or body. Complements other "ohmic" technologies such as genomics and transcriptomics to identify protein identity body and learn about the structure and function of a particular protein. State-of-the-art proteomics tools enable large-scale, high-throughput analysis for the detection, identification, and functional exploration of the proteome. Advances in protein labeling and fractionation technologies have improved protein identification to include less abundant proteins. Furthermore, proteomics has been complemented with post-translational modification analysis and methods for the quantitative comparison of different proteomes. Mass spectrometry with LC-MS-MS and MALDI-TOF / TOF as widely used equipment is the core of modern proteomics. However, the use of proteomic tools, including software for hardware, databases, and the need for trained personnel, significantly increases costs, limiting their wider use, especially in developing countries. This review attempts to describe various approaches to proteomics, recent developments and their application in research and analysis.

KEYWORDS: Proteomics, Techniques, Applications, Types, Screening.

INTRODUCTION

The dynamic role of molecules in maintaining life has been documented from the earliest stages of biological research. To demonstrate the importance of these molecules, Berzelius gave them the name *"protein"* in 1838, which comes from the Greek word *proteios*, which means *"first rank"*.[1]

Definitions

The *"proteome"* can be defined as the total protein content in a cell, characterized by its location, interactions, post-translational modifications and turnover at a given time.

The term *"proteomics"* was first introduced in 1995 and has been defined as a large-scale characterization of the complete protein complement of a cell line, tissue or organism.

Goal of Proteomics

The goal of proteomics is to gain a more complete and integrated understanding of biology by examining all proteins in a cell, not each one individually.

Using a more comprehensive definition of proteomics, many different areas of research are now grouped under the rubric of proteomics (Figure 1).

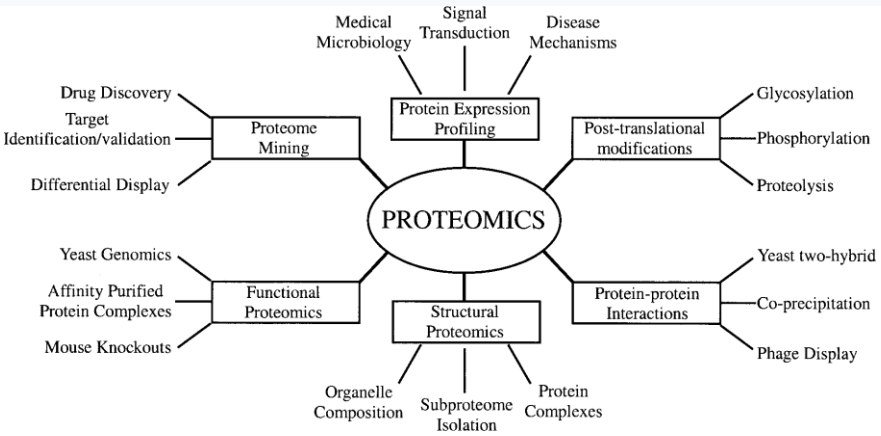

Fig. 1. Types of proteomics and their applications to biology.

Why to Study Proteomics?

Different types of information cannot be obtained by studying genes alone. For example, proteins, not genes, are responsible for cellular phenotypes. It is impossible to elucidate the mechanisms of disease, aging, and environmental effects simply by studying the genome. Only by studying proteins is it possible to characterize protein modifications and identify drug targets.[2]

Methods for Protein Screening

Conventional protein purification methods are based on chromatography, such as ion exchange chromatography (IEC), size exclusion chromatography (SEC), and affinity chromatography. For the analysis of selective proteins, enzyme immunoassay.(ELISA) and Western blot. These methods can be limited to the analysis of several individual proteins, but also cannot determine the level of expression of the protein. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis (2-DE), and two-dimensional differential gel electrophoresis (2D-DIGE) are used to separate complex protein samples.

Microarrays or protein chips have been designed for high throughput and rapid expression analysis; however, it is difficult to design a protein microarray sufficiently to investigate the function of the entire genome. Various proteomics approaches such as mass spectrometry (MS) have been developed to analyze complex protein mixtures with increased sensitivity.

X-ray Crystallography and nuclear magnetic resonance (NMR) spectroscopy are two important high-throughput techniques that provide a three-dimensional (3D) structure of a protein that can be useful in understanding its biological function.[3,4]

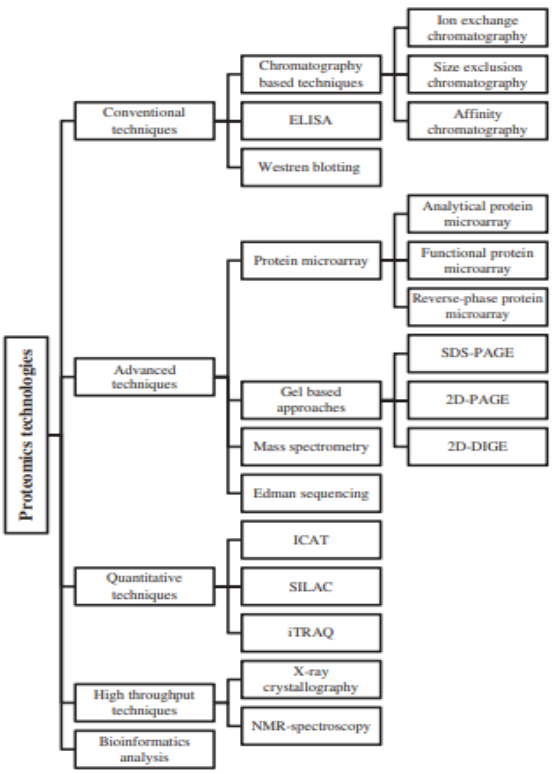

FIG. 2. An overview of proteomics techniques.

Conventional techniques for Proteomics Screening

a. Chromatography-based techniques

1. Ion exchange chromatography

It is a versatile tool for protein purification based on its load group on the surface parrot. They differ among parrots by protein in amino acid sequence; some amino acids are anionic and others are cationic. It contains a physiological pH protein which is equal to the balance of these fillers.

It initially separates proteins based on their charge nature (anionic and cationic), and then based on comparative charge strength. IEC is very valuable because of its low cost and ability to survive under damping conditions. An important virulence factor of *Helicobacter pylori* is the *neutrophil activating protein* **(HP-NAP)** which is capable of activating human neutrophils by secreting reactive oxygen species and mediators. HP-NAP is a potential diagnostic marker for H. pylori and also a potential drug target and vaccine candidate.[5]

Mussel Adhesive Proteins (MAPs) have distinctive adhesive and biocompatible properties useful in biomedical and tissue engineering. *Choi et al.* expressed recombinant MAPs in *E. coli* and was successfully purified by IEC. Antifungal proteins of the *B. subtilis* B29 strain were purified by IEC on diethylaminoethyl.

Cysteine proteases are key mediators of apoptosis and inflammation in mammals, which are expressed in *E. coli* and purified by Garica-calvo et al. for a better understanding of the catalytic properties.[6,7]

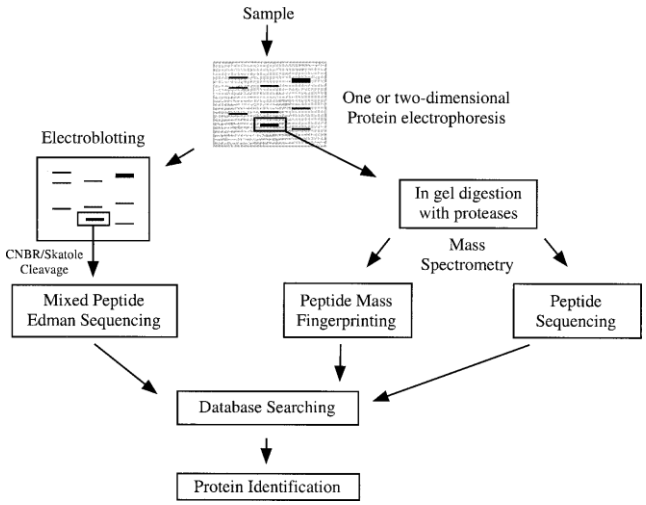

FIG. 3 Protein identification scheme. The identification of a protein in a polyacrylamide gel is described by mixed peptide sequencing or MS. For mixed peptide sequencing,

proteins are transferred to the membrane and cleaved by the CNB or skatole and the resulting peptides are sequenced simultaneously by Edman degradation. For MS, proteins are hydrolyzed by proteases and the resulting peptides are sequenced or printed. Information from all of these methods is used to search the nucleotide and protein databases to identify proteins.

2. Size exclusion chromatography

It separates proteins through a porous carrier matrix with different pore sizes depending on permeability; therefore, proteins are separated by molecular size. It is a reliable method that allows working with proteins under different physiological conditions in the presence of detergents, ions and cofactors, or at different temperatures. It is mainly used to separate low molecular weight proteins and is a powerful tool for the purification of non-covalent multimeric protein complexes under biological conditions.

Trichomonas vaginalis has the ability to damage target cells and is involved in the pathogenesis of trichomoniasis. Phospholipase-like lytic factor A2 was purified and further characterization showed two fractions of 168 and 144 kDa. The cytosolic proteins of ***Arabidopsis thaliana*** were purified to understand how the cell coordinates various mechanical, metabolic and developmental actions.[8]

3. Af*f*nity chromatography

Affinity chromatography has become a breakthrough in protein purification, allowing researchers to study protein degradation, post-translational modifications, and protein-protein interactions. The principle of affinity chromatography is as follows: ***Reversible interaction between the affinity ligand of the chromatographic matrix and the proteins to be purified.***

It has a wide range of applications for the identification of microbial enzymes primarily involved in pathogenesis. A group of amyloid-binding proteins interacts with various forms of amyloidogenic proteins and peptides, altering their pathological and physical functions.

The *homodimer and heterodimer* of HIV-I reverse transcriptase were rapidly purified by metal chelated affinity chromatography.[9,10,11]

T4 bacteriophages are free of bacterial debris and other contaminating bacteriophages. bacterial cells in "competitive phage display" produced both fusion proteins and wild-type proteins. The fusion proteins were integrated into the phage capsid and allowed efficient

purification of T4 bacteriophages. Hexahistidine affinity tags showed different affinities for immobilized metal ions, even if they both contain the same type of domain. However, zinc finger proteins differ in their biochemical properties.

b. Enzyme-linked immunosorbent assay

Engvall and Perlmann published the first paper on ELISA and the quantification of IgG in rabbit serum using the enzyme alkaline phosphatase in 1971. ELISA is a highly sensitive enzyme-linked immunosorbent assay mainly used for diagnostic purposes. This test uses antigen or antibiotics on a solid surface and the addition of enzyme-conjugated antibiotics and measures fluctuations in enzyme activities that are proportional to the concentration of antibodies and antigen in a biological sample. Diagnosis of paratuberculosis or John's disease was made possible using ethanol vortex ELISA. The analysis revealed the surface antigens of Mycobacterium avium paratuberculosis subspecies. ***Deoxynivalenol (DON)***, a potent mycotoxin produced by *Fusarium graminearum*, is an important contaminant in barley and wheat and causes Fusarium. An indirect competitive ELISA was developed for the identification of DON in wheat with a detection limit of 0.01 to 100 µg / ml in cereals. A sandwich ELISA was used to detect the Cry1Ac protein from Bacillus thuringiensis from BT transgenic cotton because its release negatively affects the environment.[13-15]

Digital ELISA is capable of detecting individual molecules in the blood. Analysis revealed serum prostate specific antigen (PSA) at a low concentration of 14 fg / ml. This analysis was able to detect ***1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (p, p'-DDE*)** a metabolite which is an insecticide and persistent organic pollutant that accumulates in the food chain and the environment.

c. Edman sequencing

Edman sequencing was developed by Per Edman in 1950 to determine the sequence of amino acids in peptides or proteins. It includes chemical reactions that remove and identify an amino acid residue present at the N-terminus of a polypeptide chain. It played an important role in the development of therapeutic proteins & quality assurance in biopharmaceuticals.

The causative agent of hemorrhagic fever, Lassa virus belongs to the Arenaviridae family. Lassa virus synthesizes glycoproteins that, after translation, are cleaved into *GP-1* (amino-terminal subunit) and *GP-2* (carboxy-terminal subunit) and are primarily involved in pathogenesis. Edman GP-2 degradation analysis reveals the N-terminal tripeptide GTF_{262}.[16]

Brucella suis survives and multiplies in macrophages by acidification. The proteins involved in this acidification have been identified. Edman digestion and comparison of 13 N-terminal amino acid sequences showed that these are signal peptides for their periplasmic localization. The *B. suis* protein, involved in membrane permeability in an acidic environment, was ***Omp25.***

The ubiquity of sesame allergy is increasing due to the consumption of baked goods and fast food. The main allergic proteins of *Sesamum indicum* were identified in allergic patients by ***2D-PAGE and SDS-PAGE*** and then analyzed by Edman sequencing. IgE-binding epitopes of these proteins have been identified that may be useful in immunotherapeutic approaches.[17-20]

Advanced techniques for Proteomics Screening

a. Gel-based approaches

1. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis(SDS-PAGE)

It is a high resolution method for separating proteins based on their size, making it easier to approximate molecular weights. Proteins are able to move under the influence of an electric field in an environment whose pH differs from the isoelectric point. Different proteins in a mixture migrate at different speeds depending on the relationship between their charge and their mass. But the addition of sodium dodecyl sulfate denatures the proteins, so they are absolutely separated based on their molecular weight. Outer membrane proteins of *E. coli* strains lacking the ability to form K1 antigen were analyzed by SDS-PAGE.[21] It showed varying degrees of sensitivity to human serum. Extracellular protein profile of Staphylococcus spp. it was also built and its characteristics were achieved. The antigenic proteins of *Streptococcus agalactiae* have been characterized to test the immunogenicity of the mastitis vaccine. The protein deposition profile in seeds and leaves of chickpea (*Cicer arientinum*) was carried out under drought conditions and without stress.[22]

Seed conservation proteins of *Brassica* species are also identified to assess the genetic divergence of different genotypes.

Cleome spp. In African countries, they are used as green vegetables and are very valuable for the treatment of coughs, fevers, asthma, rheumatism and many other diseases. Comparative analysis of leaf and seed proteins of cleome spp. was done by SDS-PAGE.

2. Two-dimensional gel electrophoresis(2D-PAGE)

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is an efficient and reliable method of separating proteins based on their mass and charge.

Proteins are separated by charge in the first dimension, and in the second, depending on the difference in their mass. 2-DE has been successfully used to characterize post-translational modifications, mutant proteins, and assess metabolic pathways. ***Neidhardt and Van Boehlen introduced the highly sensitive 2-DE method in bacteriology***.[23-25] Ongoing 2D-PAGE improvements have been used to study the metabolic system of *B. subtilis*, and the bacterial regulatory protein PyrR has been characterized.

Cell wall membrane proteins of ***Listeria innocua*** and ***Listeria monocytogenes*** involved in host-pathogen interactions were analyzed with 2-DE and 30 different proteins of the two strains were identified. This approach was useful for a comparative study of exotoxins and virulence factors secreted by enterotoxigenic strains of two foodborne *Staphylococcus aureus* strains.[26-28]

The main application of 2-DE continues to be in the protein expression profile. With this approach, the protein expression of any two samples can be compared qualitatively and quantitatively. The appearance or disappearance of spots can provide information on the differential expression of proteins, while the intensity of these spots provides quantitative information on the levels of protein expression.

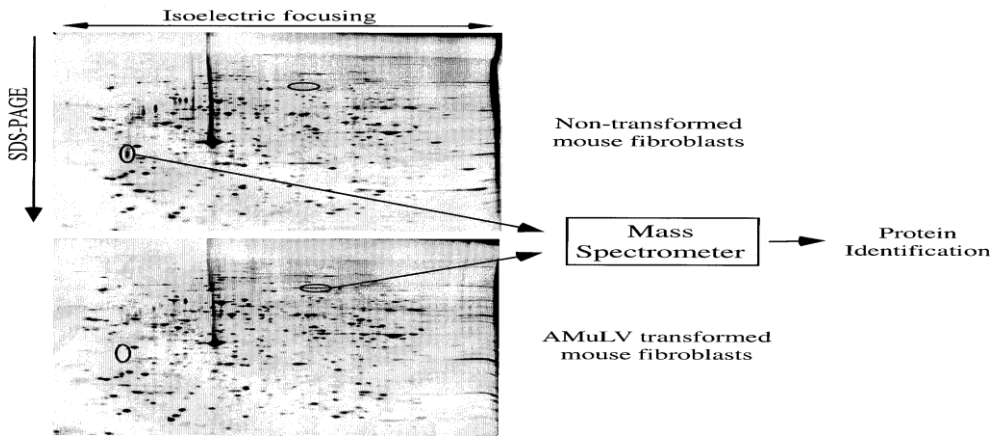

FIG. 4. Protein expression profiling by 2-DE. **Whole-cell lysates from nontransformed and Abelson murine leukemia virus (AMuLV)- transformed mouse fibroblasts were resolved by 2-DE, and proteins were visualized by silver staining. Differentially expressed proteins were excised from the gel and identified by MS.**

3. Two-dimensional differential gel electrophoresis(2D-DIGE)

2D-DIGE uses CyDye-labeled proteins that can be easily visualized by exciting the dye to a specific wavelength. Cell wall proteins labeled with Cy3 (CWP) from toxic *Alexandrium catenella* dinophagellates were identified using 2D-DIGE. About 70% of the regulated proteins showed increased expression. Proteins are also involved in regulation, adaptation to harsh environments and transport.

The plasma membrane responds to biotic and abiotic stress in plants, therefore characterization of plasma proteins provides new insight into the specific biological functions of a plant. Komatsu characterized the plasma membrane proteome of rice and *A. thaliana*.[29]

During the development of *Pinus tabuliformis* oocytes, the celization of female gametophytes is a vital process regulated by a variety of proteins, which are first extracted in anaphase and prophase and then separated by 2D-DIGE.

2-DE remains the method of choice in proteomics research, although some limitations undermine its potential as the primary separation method in modern proteomics. Thus, modern tools and techniques are rapidly expanding as a new medium for gel-free analytical methods. The development of MS in combination with shotgun proteomics can find new directions for the highly sensitive protein profile with more accurate quantification.[30,31,32]

The biological products obtained during cell culture constitute the host cell proteins (HCP) as the most important group of impurities. HCP has several molecular and immunological properties and must be effectively controlled and removed during subsequent treatment. 2D-DIGE was used to select the composition of HCP in CHO cell culture and to compare the difference in HCP between null culture and monoclonal antibody producing cells.[50,51]

b. Protein microarray

Protein microarrays, also known as protein chips, are a new class of proteomic methods that are capable of high-throughput detection of small amounts of a sample. Protein microarrays can be divided into three categories:

Analytical protein microchip

Functional protein microchip

Reverse phase protein microchip

1. Analytical protein microchip

Antibody microarrays are the most representative class of analytical protein microarrays. Once the antibodies have been captured, the proteins are detected by direct labeling of the proteins. They are commonly used to measure the level of expression and protein binding affinity. High-throughput proteomic analysis of tumor cells was performed using an antibody microarray for differential expression of proteins in oral squamous cell-derived tissues. A microchip immunoassay was used to detect staphylococcal enterotoxin *B.cholera* toxin, *Bacillus globigii* and *B. ricin.*

Mitogen-activated protein kinases (MAPKs) of Arabidopsis have been characterized. MAPKs are unique and versatile transduction molecules, highly conserved in plants, that respond to a wide range of extracellular stimuli.

2. Functional protein microarray

A functional protein microchip is created using a purified protein, which allows the study of various interactions, including protein-DNA, protein-RNA, and protein-protein, protein-drug, protein-lipid, enzyme-substrate.

The first use of a functional protein microchip was in the analysis of a specific substrate city of protein kinases in yeast. A functional protein microchip has characterized the functions of thousands of proteins. Protein-protein interaction of *A. thaliana* was studied and that identified calmodulin-like proteins (CML) and calmodulin substrates (CaM).

3. Reverse-phase protein microarray

Cell lysates obtained from various cell conditions are placed on a nitrocellulose slide, which is examined with antibodies against the target proteins. The antibodies are subsequently detected using fluorescence, chemiluminescence and colorimetric assays. For protein quantification, reference peptides are printed on slides. These microarrays are used to identify an altered or dysfunctional protein indicative of a specific disease.

Analysis of hematopoietic stem cells and primary leukemia samples using reversed-phase protein microarrays has proven to be highly reproducible and reliable for large-scale analysis of the phosphorylation status and protein expression in human stem cells and human cells. A reverse-phase protein microarray approach for quantitative analysis of phosphoproteins and

other cancer-related proteins in non-small cell lung cancer cell lines was evaluated by monitoring apoptosis, the DNA damage, cell cycle control and signaling pathways.[33-36]

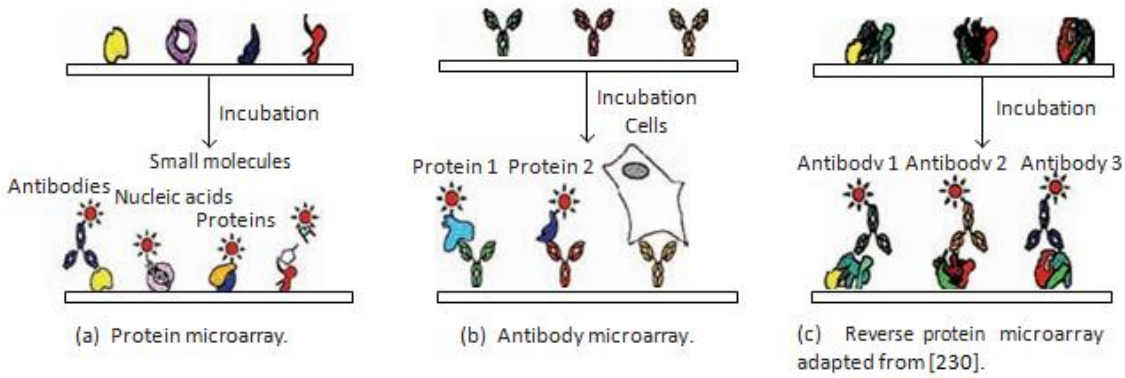

Fig. 5. Applications of functional protein microarrays and tissue array.

c. X-ray crystallography

X-ray crystallography is the preferred method for determining the three-dimensional structure of proteins. High purity crystallized samples are exposed to X-rays and subsequent diffraction patterns are processed to obtain information on the size of the repeating element, which forms crystal symmetry and crystal packing. The three-dimensional protein structure provides detailed information on how to elucidate the enzymatic mechanism, drug design, site-directed mutagenesis and protein-ligand interaction.[49]

ZipA and FtsZ are vital components of the spatial ring structure that facilitate cell division in E. coli. ZipA is a membrane-anchored protein, while FtsZ is homologous to eukaryotic tubulin, and their interaction is facilitated by the C-terminal domains. X-ray crystallography revealed the structure of the C-terminal FtsZ fragment and the FtsZ-ZipA binding complex. The movement of phospholipids, glycolipids, steroids and fatty acids between membranes is due to nonspecific lipid transfer proteins (nsLTP). The comparative structure of maize npLTP in complex with numerous ligands revealed variations in the volume of the hydrophobic cavity depending on the size of the bound ligands. Microsomal cytochrome P450 3A4 catalyzes the action of the drug - drug interactions in humans that induce or inhibit enzymes and metabolically purify drugs used clinically. The structure of the protein was analyzed by X-ray crystallography, which showed a large substrate-binding cavity capable of oxidizing huge substrates such as statins, cyclosporine, macrolide antibiotics, and taxanes.[37-40]

High-throughput techniques for Proteomics Screening

a. Mass spectrometry

MS provides structural information about proteins, such as peptide masses or amino acid sequences. This information can be used to identify a protein by searching the nucleotide and protein databases. MS is used to measure the mass / charge ratio (m / z), so it can be used to determine the molecular weight of proteins.

The general process consists of three stages. Molecules must be converted to gas phase ions in the first phase, which poses a problem for liquid or solid biomolecules.

The second step consists of separating the ions based on the m / z values in the presence of electric or magnetic fields in a compartment called a mass analyzer.[47-48]

Finally, the separated ions and the amount of each species are measured with a specific m / z value. Commonly used ionization techniques include matrix laser desorption ionization (**MALDI**), surface enhanced laser desorption / ionization (**SELDI**), and electrospray ionization (**ESI**). *Biswas and Rolain* used MALDI-TOF for the early identification of pathogenic bacteria, which is useful for early disease control. MS has also become an important tool in the study of viruses at the molecular level, and several viruses and viral proteins have been identified, including intact viruses, mutant viral strains, capsid proteins, and post-translational modifications. ***Electrospray ionization mass spectrometry (ESI-MS)*** combined with rRNA gene sequencing and PCR provided accurate and rapid identification of filamentous fungi, yeasts and medically important Prototheca species.[41]

Proteins of tomato xylem juice (*Lycopersicon esculentum*)[42] after infection with *Fusarium oxysporum* were detected by mass spectrometric sequencing and mass peptide fingerprint.

Blood proteins including IBP2, IBP3, IGF1, IGF2 and A2GL have been proposed as biomarkers for breast cancer diagnosis. MS has been used to characterize these blood proteins.

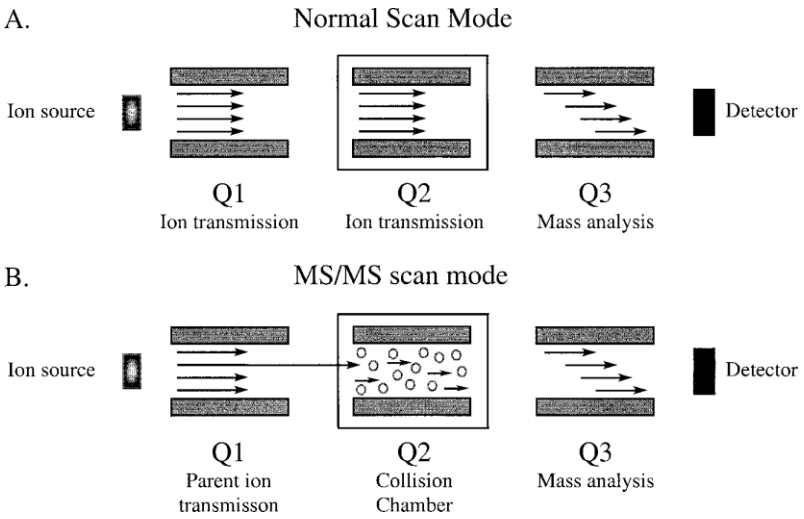

Fig. 6. MS/MS. Conventional and MS/MS modes of analysis in a triple-quadrupole mass spectrometer are shown. (A) In the normal scanning mode, all ions of a certain m/z range are transmitted through the first two quadrupoles for mass analysis in the third quadrupole. From this MS spectrum, a parent ion is selected for fragmentation in the collision cell. (B) In MS/MS mode, the parent ion is selectively transmitted into the collision chamber and fragmented, and the resulting daughter ions are resolved in the third quadrupole.

b. NMR spectroscopy

NMR is the main tool for studying the molecular structure, folding and behavior of proteins. Determination of the structure by NMR spectroscopy generally involves several steps, each of which uses a discrete set of highly specialized methods. Samples are prepared and measurements are taken, followed by interpretive approaches to confirm the structure. Protein structure is critical in several research areas, such as structure-based drug design, homology modeling, and functional genomics.

The interaction of *iso-1-cytochrome c* with yeast *cytochrome c peroxidase* was investigated by NMR. A chemical shift was observed for the ^{1}H and ^{15}N nuclei arising from the interface of enriched ^{15}N *cytochrome c* with *cytochrome c peroxidase*.

The decomposition of plant waste plays an important role in the nitrogen and carbon cycle, providing essential nutrients for the soil and atmospheric CO2. ^{15}N and ^{13}C labeled plant materials were used to monitor environmental degradation of wheatgrass and pine residues using HR-MAS NMR spectroscopy. The spectra showed that condensed and hydrolyzable

tannin is lost from all plant tissues, while the aliphatic (cuticle, wax) and aromatic (partially lignin) components are retained along with a small portion of carbohydrates.[43]

Holmes et al[54] described the differences between the metabolic phenotype of 4,630 participants from 4 human populations using NMR spectroscopy. The metabolic phenotypes included in the study were the product of interactions between various factors such as environmental, dietary, genetic and intestinal microbial activity. Selective metabolites in populations have been linked to blood pressure and urinary metabolites, opening promising new biomarker discoveries.

NMR combined with ultra-high-performance liquid chromatography (**UHPLC**) has been developed to characterize metabolic abnormalities in patients with esophageal cancer in order to identify possible biomarkers for early diagnosis and prognosis. The study revealed significant changes in ketogenesis, glycolysis, and the tricarboxylic acid cycle, as well as amino acid and lipid metabolism in esophageal cancer patients compared to controls.[44-46]

CONCLUSION

In recent years very useful advances have been made in the field of proteomics. These technologies are fast, sensitive, and have greater proteomic coverage. The combination of these technologies has resulted in success in the purification, analysis, characterization, quantification, sequential and structural analysis, and bioinformatics analysis of a large number of proteins in all types of eukaryotic and prokaryotic organisms. Areas connected to the life sciences have benefited from the increasing use of proteomic techniques. Still, more work is required for improvement of reproducibility and performance of known proteomic tools.

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