Proteome analysis by two-dimensional gel electrophoresis and mass spectrometry: strengths and limitations

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Two-dimensional gel electrophoresis, mass spectrometry and bioinformatics are the key components of current proteomics technology. The purpose of this review is to give an overview of the basic concepts of 2DE-based proteomics methodology, to discuss the advantages and limitations of the approach, and to highlight some recent advancements in 2DE-based technology.

Keywords: Mass spectrometry (MS); Protein identification; Proteome; Proteomics; Two-dimensional gel electrophoresis (2DE)

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1. Introduction

Proteins are the final products manufactured in living cells according to the 'blueprint' contained in the genome. The proteome represents the array of proteins that are expressed in a biological compartment (cell, tissue, organ) at a particular time, under a particular set of conditions. Because proteins are key structural and functional molecules, molecular characterization of proteomes is necessary for a complete understanding of biological systems.

Large-scale, comprehensive analysis of proteins is the objective of proteome science (proteomics). The scope of proteomics is broad; it encompasses: identification and quantification of proteins in cells, tissues and biological fluids; analysis of changes in protein expression in normal *versus* diseased cells; characterization of post-translational modifications; studies of protein-protein interactions; and, other applications. The goals of proteomics research include: clarification of molecular mechanisms that govern cellular processes; characterization of complex protein networks and their perturbations; discovery of biomarker proteins for detection and diagnosis of diseases; and, identification of targets for the design of drug treatments.

The expansion of proteomics was driven by technology. Although the concept of global protein analysis as a complete inventory of human proteins was proposed 20 years ago [1], proteomics research was made possible in the mid-1990s only because of concurrent developments in three areas:

1. 2DE has evolved into a robust method to rapidly separate the many proteins contained in a proteome.
2. MS methods were developed for ready and accurate analysis of 2DE-separated proteins with a high degree of sensitivity and specificity.
3. Large-scale genome research has produced a constantly increasing number of sequences that were catalogued in several databases, which could be accessed over the Internet, and search engines and other bioinformatics tools were developed to query these databases.

2DE, MS and bioinformatics tools are the key components of an approach that has been termed ''the classical proteomics methodology''. In this article, the basic concepts of 2DE-based proteomics

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methodology are described and illustrated with examples; advantages and limitations of the approach are evaluated from the perspective of an academic scientist; and, recent technical developments are discussed.

2. Basic methodology

2.1. General strategy

The basic 2DE-based proteomics methodology includes several steps (Fig. 1):

- solubilization of proteins from the sample (e.g. tissue);
- separation of the proteins by 2DE;
- digitization of 2D gels and computer-assisted analysis of protein spot patterns;
- determination of specific attributes of the proteins of interest by MS; and,
- searching of databases with these attributes to identify the proteins.

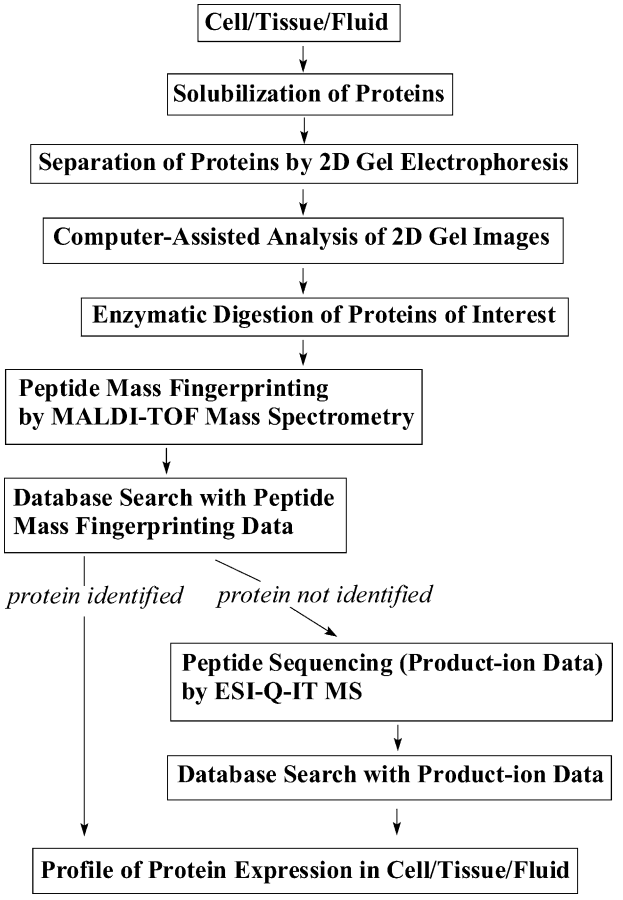

Figure 1. General strategy for proteome analysis by 2DE, MS, and database searching. Abbreviations: MALDI-ToF-MS, matrix-assisted laser desorption/ionization time-of-flight MS; ESI-Q-IT-MS, electrospray quadrupole ion-trap MS.

Treatment of samples for 2DE involves cell lysis and solubilization of proteins. Sample preparation is commonly carried out in a solution containing chaotropes, detergents, reducing agents, carrier ampholytes and, depending on the sample, protease inhibitors [2,3]. An aliquot of the sample solution is subjected to 2DE.

2DE is a powerful separation technique, which allows simultaneous resolution of thousands of proteins. The high-resolution capability of 2DE stems from the fact that the first and second dimensions are based on two independent protein characteristics. The first dimension of 2DE is isoelectric focusing (IEF), during which the proteins are separated based on their charge. In the second dimension, the proteins are separated orthogonally by SDS-PAGE according to their molecular weight (MW).

2DE was first introduced in the early 1970s [4]. However, its widespread application was hampered by experimental drawbacks and by the lack of techniques capable of analyzing 2DE-separated proteins.

The experimental difficulties were overcome by the introduction of immobilized pH gradient gels for IEF that are now commercially available. Using these standardized gels, it is now possible to separate higher loads of proteins sufficient for further characterization and to generate highly-reproducible 2D maps. After separation, proteins in 2D gels are visualized by staining, commonly with a Coomassie Blue stain, or with a modified silver stain that is compatible with subsequent MS analysis [5]. The 2D gels are digitized and the resulting gel images are qualitatively and quantitatively analyzed with specialized software programs. In this manner, proteins can be quantified and spot patterns in multiple gels can be matched and compared. Statistical analysis can be performed on groups of features (spots) in sets of gels, and variations, differences, and similarities can be evaluated.

Proteins resolved by 2DE can be identified based on unique attributes that are measured by MS. These attributes are determined from analysis of peptides generated by proteolytic digestion of the protein of interest. The most commonly used enzyme for protein digestion is trypsin, which cleaves the protein at the C-terminal side of lysine and arginine. If needed, proteases with other specificities can also be employed.

Two specific protein attributes can be obtained by MS analyses of proteolytic digests. The first protein attribute is the so-called peptide-mass fingerprint. Peptide-mass fingerprinting involves determination of the masses of all peptides in the digest. The second attribute includes fragmentation of selected peptides inside the mass spectrometer into series of sequence-diagnostic product ions. From these product ions, a portion of the amino acid sequence of the peptide (a 'sequence tag') can be deduced; alternatively, uninterpreted product-ion spectra can be used directly for protein identification.

MS instrumentation techniques that have played a key role in proteomics and in the analysis of peptides and proteins in general are matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-ToF-MS) and electrospray ionization-quadrupole ion trap MS (ESI-Q-IT-MS). MALDI-ToF-MS and ESI-Q-IT-MS are based on different physicochemical principles and have different characteristics [6]; therefore, they can yield complementary analytical information. These two methods provide the needed sensitivity and specificity for proteomics research.

MALDI-ToF-MS is commonly used for peptide-mass fingerprinting. The method is user-friendly, fast (3–5 min per analysis), sensitive (fmol peptide levels), and it measures peptide masses with accuracy better than 50 ppm. However, MALDI-ToF-MS is impractical for the sequencing peptides in proteolytic digests, unless the peptides are derivatized prior to analysis [7].

To obtain peptide-sequence data (product-ion spectra), ESI-Q-IT-MS is usually employed; typically, peptide mixtures are first separated by liquid chromatography, which is coupled on-line to the ESI-Q-IT mass spectrometer. This method requires about 1h per analysis, but good-quality product-ion data are a very specific attribute for protein identification. Many proteomics laboratories have adopted a two-tiered protein-identification strategy (Fig. 1), where MALDI-ToF-MS is used first to obtain peptide-mass fingerprinting data, and a database search is performed with these data. If the protein is not unambiguously identified, then the more time-consuming ESI-Q-IT-MS analysis is carried out to generate product-ion data.

Peptide-mass fingerprints, product-ion data or peptide-sequence tags are used to search a protein-sequence database to identify the protein of interest [8]. The identification is made by comparing the experimentally-generated data with theoretical data calculated for each database entry. The rationale is to retrieve proteins that would produce the same set of data if digested and analyzed in the same manner as the protein under study. Usually, a list of candidate proteins that most closely match the input data is generated by the search, and the candidate proteins are ranked using various scoring algorithms. Constraints can be included to limit the search to a specific subset of database entries, e.g., proteins from a particular species. Several protein-sequence databases are available in the public domain. An excellent annotated database is the SWISSPROT database that is maintained by The Swiss Institute of Bioinformatics and The European Bioinformatics Institute [9,10]. The main advantages of the SWISSPROT database are low redundancy and a high degree of annotation. The August 2002 release (Release 40.25) of the database contains 112,657 protein entries.

Database search programs are often included in commercial software packages that are provided with mass spectrometers. One such example is the SEQUEST program that is used for database searching with uninterpreted product-ion spectra. A number of search engines can also be accessed free-of-charge over the Internet, for example the PeptIdent and MultIdent programs at the ExPASy Molecular Biology server [11], MS-Fit and MS-Tag at the Protein Prospector server [12], or MASCOT at the Matrix Science server [13]. These websites also provide additional proteomics software tools, technical information, and links to other resources.

An important set of web-based resources are reference databases of proteins identified by 2DE-based proteomics. In recent years, such reference databases have been established for various tissues, cell lines, body fluids and other biological systems. A reference database usually contains one or more 2D-gel images and textual information about identified proteins that can be retrieved by clicking on a particular spot on the gel images. Guidelines have been proposed in an effort to standardize the building of 2DE reference databases [14]. An index of 2DE databases that are available to the scientific community can be accessed at the ExPASy proteomics server [11].

2.2. Example of proteome analysis

In this section, data from a proteome analysis of human-prostate tissue are shown to illustrate the 2DE-based proteomics approach and some of the typical results. Proteins from a whole prostate-tissue specimen were solubilized, separated by 2DE, and visualized by staining with Coomassie Blue. The 2D map of the human-prostate proteome is shown in Figure 2; approximately 400 protein spots of various intensities are detected in this map. Protein-identification data for 10 representative spots (labeled with numbers in Fig. 2) from the prostate proteome are summarized in Table 1. The proteins in these spots were identified based on peptide-mass fingerprinting or product-ion data that were determined by MS analysis of tryptic digests.

An exemplary MALDI-ToF mass spectrum is depicted in Fig. 3. The spectrum contains 24 peptide ions with masses of 0.5–2.6 kDa that represent the peptide-mass fingerprint of the protein in spot 10. Several ions arising from trypsin autodigestion (labeled T) are also seen in the spectrum. (Because they appear at known masses, trypsin-autodigestion peaks are commonly used for internal calibration of the mass scale to enhance the accuracy of mass measurement). Based on these peptide-mass fingerprinting data, the protein in spot 10 was identified as human serotransferrin.

Fig. 4 shows a typical product-ion spectrum obtained by ESI-Q-IT MS analysis of protein spot 6. It should be noted that the tryptic digest was first separated by capillary HPLC, and each eluted peptide was introduced on-line into the mass spectrometer and analyzed. Thus, a product-ion spectrum was recorded for each of the tryptic

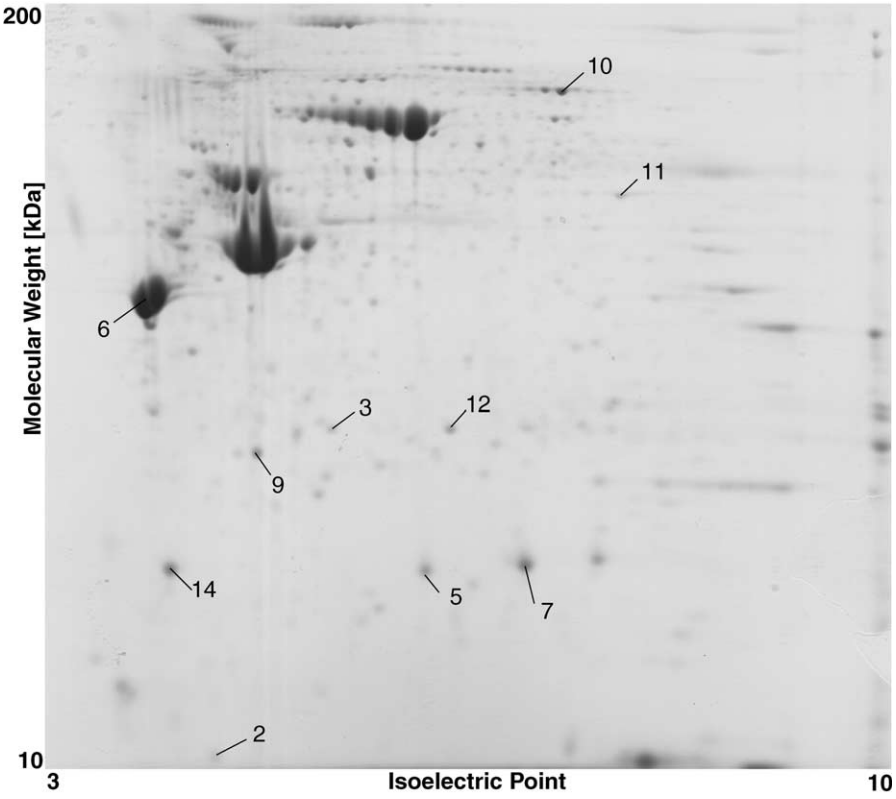

Figure 2. 2D map of the human-prostate proteome. To produce this map, ca. 600 μg of protein from a whole tissue homogenate were separated by 2DE, and the proteins were detected by staining with colloidal Coomassie Blue. Proteins identified by MS are labeled with numbers.

Table 1. Identified proteins from the human prostate proteome

Spot No.	Protein name	SWISSPROT Accession Code
2	Galectin-1	P09382
3	Heat Shock 27 kDa Protein	P04792
5	Transgelin	Q01995
6	Tropomyosin Beta Chain	P06468
7	Transgelin	Q01995
9	Apolipoprotein A-I	P02647
10	Serotransferrin	P02787
11	Alpha Enolase	P06733
12	Heat Shock 27 kDa Protein	P04792
14	Myosin Regulatory Light Chain 2	P24844

peptides. (In contrast, in the MALDI-ToF experiment described above, a single spectrum was produced from all peptides in the tryptic digest). The spectrum in Fig. 4 displays series of product ions that arise from gas-phase fragmentations of the particular peptide and that are diagnostic of the peptide's sequence. All product-ion spectra generated in the ESI-Q-IT analysis of the digest of spot 6 were used in a database search to pinpoint the sequences of the peptides and to identify the protein in spot 6 as human tropomyosin.

3. Advantages

In recent years, the combination of 2DE, MS, and bioinformatics tools has been utilized extensively for proteomics research in industry and academia. The power of the 2DE-based technology was recognized by the research community early on, and scientists from various disciplines were attracted to the field of proteomics. As many proteomics projects got under way, limitations of the 2DE-based technology became increasingly apparent. To overcome these limitations, a large effort has been focused on improving the capabilities of 2DE-based proteomics. In addition, alternative approaches that circumvent 2DE were developed, such as global quantification of proteins using isotope-coded affinity tags [15] or the combination of accurate mass tags and Fourier transform ion cyclotron resonance (FT-ICR)-MS [16], or protein expression profiling with surface-enhanced laser desorption/ionization [17]. These alternative methodologies significantly expand the spectrum of tools available for proteomics research. Nevertheless, the 2DE-based approach has several characteristics that are currently unmatched by other proteomics methodologies:

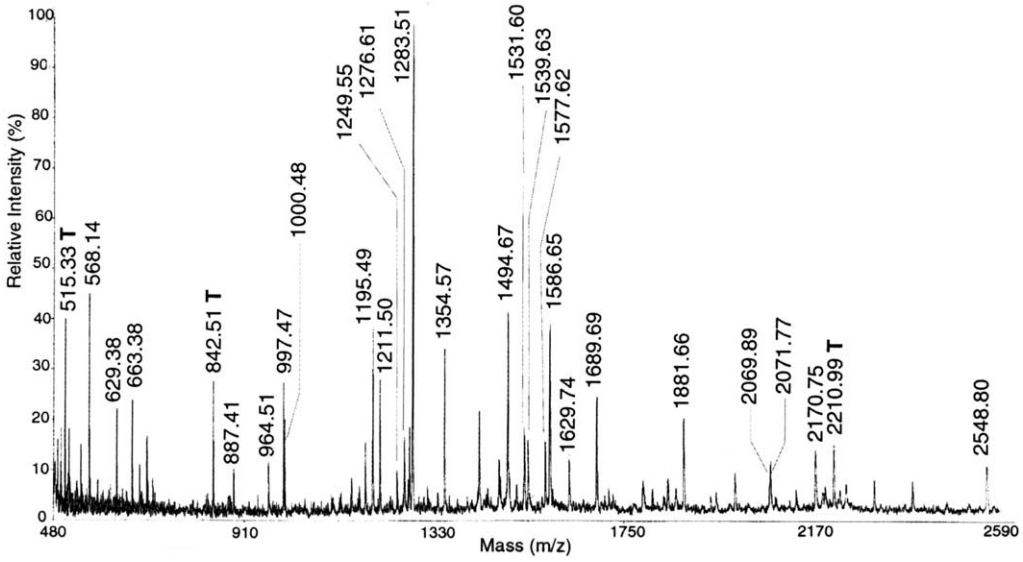

Figure 3. MALDI-ToF mass spectrum of the tryptic digest of protein spot 10 from the human-prostate proteome. The letter T indicates trypsin-auto-digestion peptides.

expected productions

Seq	#	b	y	(+1)
C	1	161.0	-	9
A	2	232.0	960.5	8
E	3	361.1	889.4	7
L	4	474.2	760.4	6
E	5	603.2	647.3	5
E	6	732.3	518.3	4
E	7	861.3	389.2	3
L	8	974.4	260.2	2
K	9	-	147.1	1

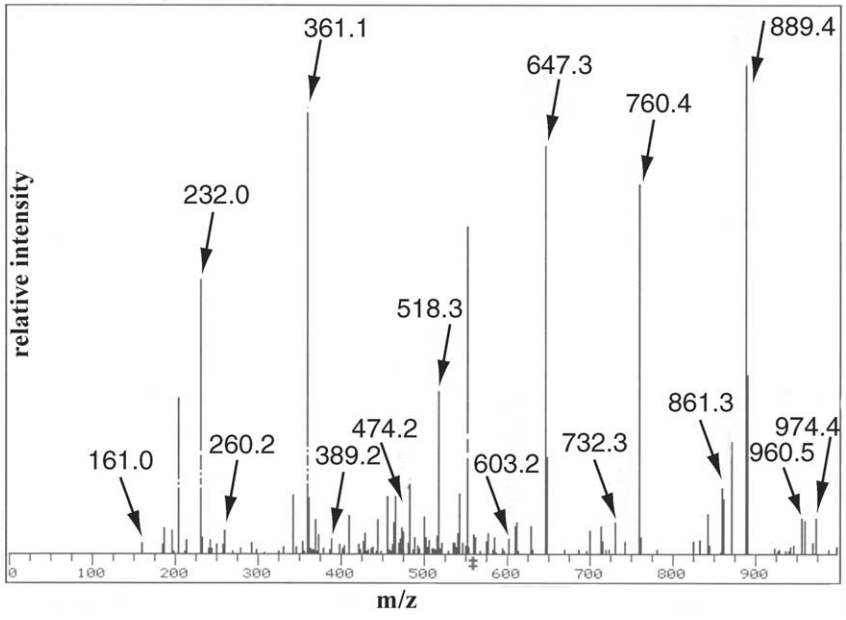

Figure 4. Product-ion spectrum of the peptide C*AELEEELK (where C* denotes carbamidomethylcysteine) from the tryptic digest of protein spot 6 from the human-prostate proteome. The data were obtained by ESI-Q-IT-MS.

1. The information content of the data obtained by the 2DE-based approach is high because a number of specific protein attributes can be determined. Thousands of proteins can be resolved and visualized simultaneously on a single 2D gel; for each protein, the isoelectric point, MW, and the relative quantity can be measured. With MS, each protein can be characterized *via* a unique peptide-mass fingerprint and/or amino-acid sequence tag.
2. High-resolution capabilities of 2DE allow the separation and detection of post-translationally modified proteins. In many instances, post-translationally modified proteins can be readily located in 2D gels because they appear as distinctive horizontal or vertical clusters of spots. In addition, modified proteins can be revealed by MS analysis, when multiple spots of the same protein are identified.

3. Individual steps of the proteome analysis (2DE, imaging, MS, database searching) can be separated in space and time. For example, 2DE and computer-assisted analyses of spots patterns can be performed in an investigator's laboratory, and protein spots of interest can be analyzed in a service facility at a later date. It is also important to point out that 2D gels can serve as high-capacity 'fraction collectors' for the purification and long-term archival of proteins. On a single, dried 2D gel, thousands of proteins can be stored at room temperature, in a space equal to one notebook page. In this manner, proteins from precious sources, such as rare tumor-tissue specimens can be preserved for extended periods of time. Proteins stored within dried gels for months, even years, can be identified by MS.
4. In terms of equipment and personnel resources, the 2DE-based technology is well suited for research conducted in an academic setting. Most scientists engaged in biological research are familiar with one-dimensional gel electrophoresis; 2DE, while more complex and labor-intensive, is a natural extension of their expertise. In addition, 2DE equipment is relatively inexpensive and can therefore be supported by individual project grants. Access to other essential components, such as mass spectrometers and bioinformatics resources, can be obtained through shared-instrumentation and/or fee-for-service facilities, which are in place at many academic institutions. Thus, many investigators from various scientific disciplines can incorporate proteomics into their research programs. By contrast, alternative proteomics methodologies [15,16] rely almost exclusively on cutting-edge, high-cost MS instrumentation. For example, a commercial state-of-the-art FT-ICR mass spectrometer can cost over $1m, is expensive to maintain, and requires expert operators. Because of these considerations, FT-ICR technology is, for the time being at least, simply inaccessible to most investigators.

4. Limitations

The greatest challenge for proteomics technology is the inherently complex nature of cellular proteomes. Many factors contribute to proteome complexity. Unlike a genome, a proteome is a highly dynamic entity. Protein expression in a biological system changes with the state of development, in response to environmental stimuli, with the progression of a disease, etc. In addition, different cells within a multi-cellular organism have different proteomes. The number of proteins in a proteome is very large. Although no precise calculations can be made, it is estimated that up to 50,000 protein species may be simultaneously present in a eukaryotic cell [18]. The dynamic range of protein expression spans seven or eight orders of magnitude and, consequently, proteins are present in vastly different quantities. Furthermore, proteins within a proteome are structurally diverse and have various physicochemical characteristics. Because of these considerations, comprehensive characterization of cellular proteomes is an enormous undertaking. 2DE in combination with MS plays a central role in proteomics. However, several limitations and problem issues have been recognized:

1. The most important drawback of 2DE-based proteomics is that it is not possible to analyze the entire proteome. Proteins displayed in a single 2D gel represent only a portion of all the proteins that are present in a sample. Generally, proteins that are visualized in 2D gels by conventional staining methods are high-abundance proteins. The limit for the detection of proteins with silver staining is approximately 1 ng, (i.e., 20 fmol for a 50 kDa protein) [5]. Low-abundance proteins, which are not detected by conventional staining, include regulatory proteins, receptors, and other proteins that play key roles in cellular processes. Besides low-abundance proteins, other groups of proteins with specific properties are difficult to analyze by 2DE, and are generally not observed under standard conditions. These proteins include very small and very large proteins, alkaline proteins, and hydrophobic proteins. One particular class of proteins that is not readily amenable to 2DE are membrane proteins [19]. Major limiting factors for 2DE analysis of membrane proteins are their poor solubility in standard protein extraction solutions, and their generally low abundance.
2. The accuracy of the quantification of proteins in silver-stained gels is compromised by the narrow dynamic range of the stain, and by the fact that different proteins have different staining characteristics. Furthermore, because of the complexity of the silver-staining procedure, reproducible spot intensity is difficult to achieve. Coomassie Blue stain is more suitable for protein quantification, but the sensitivity of Coomassie Blue stain is significantly lower than the sensitivity of silver staining.
3. 2DE is labor-intensive and has a relatively low throughput. The throughput of 2DE is adequate for many basic research studies, but it may present a serious obstacle for projects that involve screening of a large number of clinical samples,

or for any other research where high throughput is critical.

4. MS analysis of proteins from silver-stained 2D gels is not routine. Silver stain detects proteins down to the fmol level and, consequently, the quantity of protein in a silver-stained spot is usually low. The amount of sample available for analysis is further reduced through losses that occur during the preparation of peptide digests. MS data from digests of silver-stained proteins may contain only a few peptide signals, which may not be enough for unambiguous protein identification. Because of the low amounts of the analyte, analysis of proteins from silver-stained gels is also more susceptible to interferences from sample contamination. One particularly bothersome class of contaminating proteins is keratins from human skin and hair that can create a serious problem for MALDI-ToF-MS.

5. Recent advancements

A number of modifications to the 2DE-based methodology have been introduced and explored, and improvements in the key components of the technology have been achieved. Some of the recent advancements are discussed in this section. However, it should be emphasized that there is no universal protocol that would be generally applicable to all proteomics research.

Before starting a proteomics study, the advantages and disadvantages of various methods must be assessed in order to choose the best suitable approach. The choice of an appropriate methodology will depend on the goals of the specific study, availability of resources, and other factors.

One consideration that is important for the design of all proteomics studies is the issue of sample availability. There is no equivalent to PCR for protein amplification and, therefore, all protein material must come directly from the sample source. Consequently, the amount of protein that is available for analysis will determine the optimum approach; a combination of methods appropriate for analyses of cells that can be cultured in large quantities will most likely be different from methods suitable for analyses of biopsy tissues, where only a limited amount of protein can be obtained.

5.1. Pre-fractionation

Pre-fractionation of proteins prior to 2DE separation can be carried out to reduce the complexity of the protein mixtures and/or to isolate specific sub-sets of proteins. Several types of sample pre-fractionation techniques have been developed. Sequential extraction with a series of buffers of different solubilization power can be used to fractionate proteins based on their relative solubilities, for example to enrich for membrane proteins [20]. Protein samples can also be pre-fractionated based on their sub-cellular localization to separate proteins from different cellular organelles or compartments [21]. Another pre-fractionation approach utilizes liquid-phase IEF to separate proteins based on their charge [22,23]. Complex proteomes can also be simplified by reversed-phase HPLC [24] or ion exchange chromatography [25]. Enrichment and/or removal of specific proteins can be accomplished by affinity chromatography [26–28].

5.2. Laser capture microdissection

An important issue relevant to sample preparation for proteomics is procurement of targeted cell populations, e.g. tumor cells, from tissue specimens. The methods commonly employed to prepare enriched tumor samples involve extraction of tumor cells by fine-needle aspiration or by scraping of frozen tissue sections.

Recently, laser capture microdissection (LCM) technology has been introduced to enable the isolation of pure cell populations [29]. The compatibility of LCM with 2DE-based proteomic analysis of human tumors has been demonstrated [30]. At present, a major drawback of this approach is that a large number of cells must be microdissected to obtain enough material for 2DE analysis [31]. Nevertheless, LCM is a powerful technique that holds great promise for proteomics. Many proteome studies currently under way use tissue specimens, and these studies are likely to benefit from LCM in the future, as more sensitive techniques for the detection of 2DE-separated proteins become available.

5.3. Immobilized pH gradient strips

Introduction of immobilized pH gradient gels (IPG strips) for IEF has played a major role for the widespread application of 2DE. Recent advancements include improvements in the separation of alkaline proteins, and design of narrow-range IPG strips covering a single pH unit [32]. At present, a large variety of IPG strips of assorted lengths and pH ranges are commercially available.

Wide-range IPG strips, e.g., pH 3–10, give an overall overview of the expressed proteins, but they do not provide the spatial resolution needed for efficient separation of proteins in complex proteomes. This results in co-migration and overlapping of proteins with similar electrophoretic properties, and that hampers protein detection, quantification and identification.

One solution to this problem involves the use of a series of medium-range or narrow-range pH gradient strips [33]. However, this strategy, which increases sample requirements and offers lower throughput, may not be practical for many proteomics studies.

5.4. SDS-PAGE systems

Horizontal SDS-PAGE units in combination with ready-made gels and buffer strips are commonly used for the second dimension of 2DE. However, vertical SDS-PAGE systems may be more suitable for high-throughput comparative proteomics. With vertical systems, multiple SDS-PAGE separations can be run in parallel, and the quality of the resulting 2D-spot patterns is superior to that obtained with horizontal systems. Reproducible, high-quality 2D patterns are essential for the success of computer-assisted 2D-gel analyses.

5.5. Fluorescent dyes

Improved techniques for the detection of proteins in 2D gels involve staining with fluorescent dyes, such as SYPRO Ruby. Fluorescent dye stains are well suited to 2DE-based proteomics and offer several advantages over traditional staining methods [34]. The sensitivity of the SYPRO Ruby stain is comparable to that of silver staining. In contrast to silver stain, the SYPRO Ruby stain has a broad dynamic range, which allows accurate protein quantification. Staining with SYPRO Ruby is also compatible with MS methods for protein identification.

An elegant modification of 2DE, called difference gel electrophoresis (DIGE), which utilizes labeling with fluorescent dyes, has been developed [35,36]. In this approach, proteins in two sample populations are tagged with different fluorescent dyes so that only one gel is needed to separate the proteins and to quantify differences between the two sample populations. The method offers a higher throughput that conventional 2DE, and eliminates the gel-to-gel variations in protein migration that hinder computer-assisted comparison of spot patterns. A DIGE-based technology is commercially available [37].

5.6. Robotics

Robots for the excision of protein spots from 2D gels, and modules for automated protein digestion have been developed to expedite the identification of proteins in proteomics. These robotic systems are often interfaced with image analysis software and with MS instrumentation. The use of spot-picking and protein-handling robots not only increases the speed of the analysis but also minimizes contamination of the samples with keratins that interfere with high-sensitivity protein identification.

5.7. MS

MALDI-ToF-MS remains an important tool for protein identification because of its high throughput, sensitivity, and high mass accuracy. User-friendliness and moderate cost of the instrumentation make MALDI-ToF an attractive technology for academic laboratories. Numerous advancements have been made in MALDI-ToF instrumentation and new-generation, automated MALDI-ToF mass spectrometers are commercially available. These high-throughput systems are run without operator intervention, and incorporate algorithms for iterative optimization of instrument parameters during data acquisition. Improved software tools for the detection of monoisotopic peaks in MALDI-ToF spectra have also been developed.

Another type of newly developed MS instrumentation combines electrospray ionization (ESI) with a quadrupole time-of-flight (QToF) analyzer [38]. The ESI-QToF instrument is excellent for high-sensitivity sequencing of peptides in proteolytic digests of 2DE-separated proteins. It has been demonstrated that high-quality product-ion spectra can be obtained for peptides derived from digests of low-level proteins from silver-stained 2D gels [39]. The QToF analyzer can also be coupled with MALDI, and MALDI-QtoF-MS was shown to be a promising new tool for proteomics [40]. Recently commercialized versions of the QToF mass spectrometer include both ESI and MALDI sources, and the instrument can be easily configured for either ESI-QToF or MALDI-QToF operation.

The latest generation of proteomics instrumentation also includes the MALDI tandem-time-of-flight (MALDI-ToF/ToF) mass spectrometer [41]. The major advantages of the MALDI-ToF/ToF instrument are ultra-high throughput, high sensitivity, and high-energy collision-induced dissociation capabilities that provide enhanced peptide-sequence information. Furthermore, both peptide-mass fingerprinting and peptide-sequencing data can be recorded on a single instrument, using the same portion of a sample (such as a proteolytic digest).

6. Conclusions

Since the introduction of proteomics, 2DE and MS have been successfully used in a large number of studies in many biological fields. Recent advancements have significantly enhanced the capabilities of the 2DE-based technology, and it is likely that 2DE will remain an important tool for proteomics research in the near future. Further technical improvements are needed to enable the detection and characterization of low-abundance proteins, to improve protein-quantification capabilities, and to increase the throughput of the methodology.

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