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Review

Two-dimensional gel electrophoresis in the light of new developments

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ABSTRACT

Two-dimensional gel electrophoresis (2-DE) has become the most widely used separation tool in proteomic analysis. 2-DE is especially useful in expression proteomics, where comparative analysis of the expression of proteins exposed to environmental factors and those physiologically undisturbed is the essence of research. A comparative analysis of changes in protein expression provides information that may be utilized (e.g., in drug design and biomarker research). Furthermore, the technique of 2-DE is gaining popularity due to the possibility of coupling it with numerous analytical techniques and bioinformatics methods.

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1. Introduction

In the twenty-first century, we clearly need to understand the relationship between the phenotype and the genotype of an organism. Understanding the nature of cancer and cell mutations is a challenge for the modern science. It is well known that all biological processes are controlled by proteins, which are the essential organic compounds present in living organisms. They make the fundamental biologically-active agents, such as enzymes, hormones, and antibodies. Not only do they determine the structure of the system, the proper functioning and the development of living organisms, but they also control every cellular process, including the processes of DNA replication and RNA transcription,

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protein translation and splicing [1,2]. For this reason, the need for a new field of science that uses proteins to describe the state of an organism, was born, so, in 1994, Wilkins [3] called it "proteomics".

Proteomics deals with the study of proteins, temporal expression and determination of biological structures, functions and interactions. The terms "proteomics" and "proteome" refer to the previously introduced terms "genomics" and "genome", describing the complete set of genes of a cell or an organism. The suffix "omics" in these words symbolizes a new philosophy of action for the overall understanding of the functioning of living systems [4,5]. Characterization and identification of proteins encoded by active genes is the aim of proteomics. The population of proteins present in a system (cell, tissue, or body fluids) in a given time is referred to as the proteome. There is no direct relationship between the level of gene expression and protein expression. This means that the number of proteins is much greater than the number of genes encoding them, because of the modifications that proteins undergo. However, the presence of PTMs, alternative splicing, or modifications caused by environmental factors resulting in increased proteome dynamics in relation to the genome are also important. The development phase, the pH or the temperature influence the expression of different proteins in different tissues. The ultimate goal of proteomics is to generate protein profiles, compare them and detect differences between them [2,4–6].

Specific proteins are indicators of the activity of an organism. Protein studies require the use of analytical technology characterized by high selectivity, resolution and sensitivity. Success in research can be measured when using high-resolution proteomic electromigration techniques. The use of proteomic analysis can be relatively quick in searching for new cancer biomarkers, which will be used in future for both screening and prognosis of the disease. Until recently, diagnostics based on one marker for the diagnosis of a large and heterogeneous patients group resulted in loss of sensitivity and accuracy. Proteomic studies allow application of several protein markers at a given time. Proteomics also favors more accurate diagnosis of a disease, development of new types of treatment depending on the individual needs of the patient and better monitoring of the response to the treatment [6]. Thanks to proteomics, it is also possible to understand better the mechanisms of diseases, to facilitate development of new drugs and vaccines, and to make a detailed study of pathogens.

The study of the proteome can be divided into three main stages: acquisition of the material and its pre-treatment, specific protein profile analysis and analysis of the obtained data. Because the proteome is a dynamic structure, getting the appropriate material to be analyzed and its proper storage is a critical step for proteomic analysis. One of the problems is isolation of diseased cells from a fragment of a tissue. In order to separate the sick cells precisely from the healthy ones, scientists use the relatively new technique of tissue preparation via laser dissection through a microscope. Despite the difficulties in analysis and processing of massive amounts of information obtained in clinical proteomics, study of the proteome can be successful, thanks to the use of high-resolution spectrometers and bioinformatics methods [2,5,6].

Due to the enormous complexity of the tested material, the use high-resolution separation techniques is of prime importance. The study of the proteome is typically preceded by electrophoretic separation of a protein mixture. Proteomic studies thus include protein separation, identification, quantitative measurements, sequence analysis (bioinformatics), the study of structure, and, finally, the study of interactions and modifications of proteins.

Two-dimensional electrophoresis (2-DE) is most important in the search for cancer biomarkers. 2-DE was first described in 1975 independently by O'Farrell and Klose [7]. Since the first publication, the use of 2-DE has attracted constantly growing interest.

2. Preparation of biological material for electrophoretic analysis

The proteome is a dynamic structure, so the sample itself and the conditions of storage of the material for research directly affect the result of proteomic analysis. The first step is to prepare biological material with precision and repeatability of performance, using ultrapure reagents. A general scheme of sample preparation should include disintegration of cells (tissue), protein extraction, purification and reconstitution in a proper buffer. Because of the activity of proteolytic enzymes and destructive oxidation processes, the sampled biological material should not be stored at room temperature (RT). Fractionated samples are to be placed in dry ice (−78.5°C) or liquid nitrogen (−198°C) immediately after collection. Isolation of proteins is carried out by disintegrating cells: mild and strong lysis (Table 1). [8].

During the process of cell lysis, endogenous proteases are released. In order to inactivate the enzymes, quick freezing in liquid nitrogen, denaturation of the sample (for example, 10% TCA, 8M urea or 2% SDS), or application of specific protease inhibitors (e.g., EDTA, chloromethyl ketone, or benzamidine) should be used.

A further step is disintegration of the cellular analyte and purification of proteins from contaminants, such as salts, nucleic acids, polysaccharides, lipids, detergents, ionic species, and phenols [8–17] (Table 2).

The protein-purification process is followed by protein dissolution in a chosen buffer. The role of the buffer is to denaturate proteins and to prevent their aggregation [8–19] (Table 3).

Determination of protein concentration in the analyzed sample is the next step. The most popular methods are those created by Lowry and Bradford [20–22].

Table 1
Types of cell disintegration

Type	Feature	Material	Ref.
Cell lysis in mild conditions			
Osmotic lysis	Cells in liquid suspension, hypoosmotic	Blood cells, tissue cultures	[4,16]
Freezing and thawing	Repeated quick freezing in liquid nitrogen followed by thawing	Bacterial cells, plants, animal tissues	[4]
Detergent lysis	Ionic: SDS; non-ionic: Triton X; zwitterionic: CHAPS	Bacterial cells, plants, animal tissues	[4,18]
Enzymatic lysis	Specific enzymes:		[2,4,16,18]
	A) Lysozyme	A) Bacteria	
	B) Cellulase	B) Plants	
	C) Glucanase	C) Yeast	
Cell lysis in harsh conditions			
Sonication	The use of ultrasonic waves	Animal tissues, bacteria, plants	[4,16]
High-pressure	French press	Bacteria, fungi, algae, plants	[4,14]
Grinding	Triturating with frozen sand or alumina powder	Bacterial cells, plant, animal tissues	[12]
Mechanical homogenization	The use of a blender	Soft animal tissues	[16]
Shake with glass beads	Mechanical damage due to contact with suspended glass beads	Bacterial cells, plants, animal tissues	[14]

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