Acta Tecnología – International Scientific Journal about Technologies
Volume: 4 2018 Issue: 2 Pages: 25-28 ISSN 2453-675X

doi:10.22306/atec.v4i2.35
Received: 16 May 2018
Accepted: 30 May 2018

BASIC PRINCIPLES OF SAMPLE PREPARATION FOR PROTEOMIC ANALYSIS

Marianna Trebuňová
Technical University of Košice, Faculty of Mechanical Engineering, Department of Biomedical Engineering and Measurement, Letná 9, 042 00 Košice, Slovakia, marianna.trebunova@tuke.sk (corresponding author)
Jozef Živčák
Technical University of Košice, Faculty of Mechanical Engineering, Department of Biomedical Engineering and Measurement, Letná 9, 042 00 Košice, Slovakia, jozef.zivcak@tuke.sk

Keywords: proteins, proteomics, sample preparation
Abstract: Proteomics studies and evaluates all proteins found in cells, tissues, organisms in terms of quantity, structure, function, and their interaction. An important step in this discipline is the preparation of the studied sample. Proteomics evaluates the samples obtained from patients from body fluids and tissues, this step is the starting point of the whole methodology.

1 Introduction, analysis of proteomics samples

Proteomics studies and evaluates all proteins found in cells, tissues, organisms in terms of quantity, structure, function, and their interaction. An important step in this discipline is the preparation of the studied sample. Proteomics evaluates the samples obtained from patients from body fluids and tissues, this step is the starting point of the whole methodology [1].

The main goal of the proteomic approach is global protein analysis. To achieve this goal, methods such as 2D electrophoresis, mass spectrometry type - electrospray ionization, matrix-assisted laser desorption ionization associated with flight time detection and fragment detection by tandem mass spectrometry are used.

In order to get the right results in the study of proteins using the techniques already mentioned, the first step is the correct preparation of a sample obtained from the patient, Figure 1.

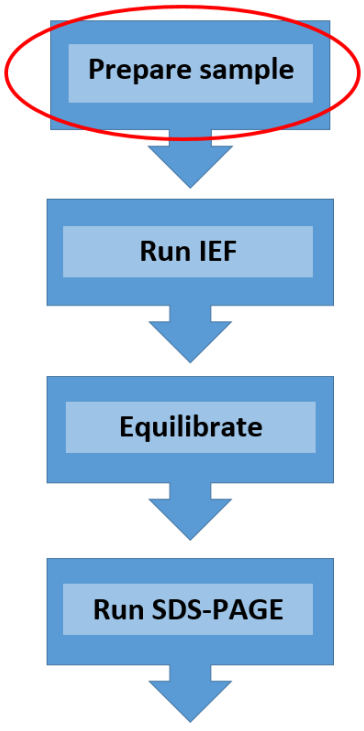

Figure 1 Schematic part of proteomics

2 Sample preparation

Sample preparation by proteomic analysis consists of the individual steps that are shown in the diagram, Figure 2.

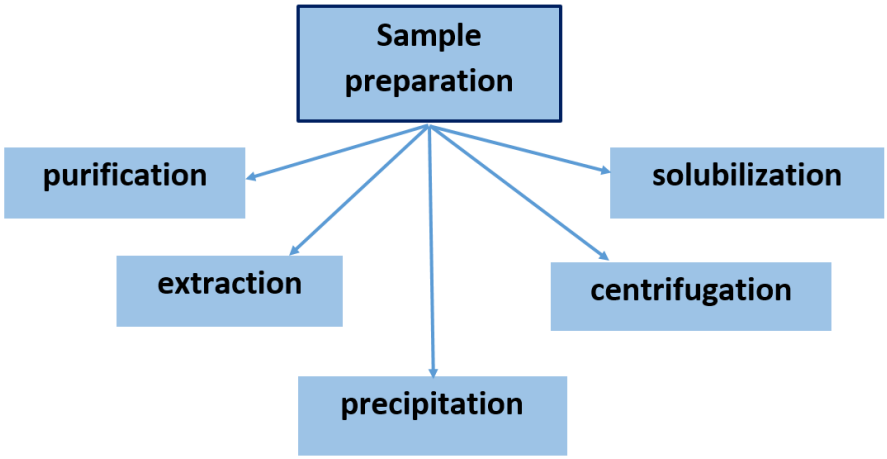

Figure 2 Sample preparation in individual steps

2.1 Purification

Protein purification is a series of processes intended to isolate one or more proteins from a complex mixture, usually cells, blood, plasma, serum or parts of tissue. Protein purification is important for characterizing their function, structure and protein interactions. In this process, protein and non-protein can be separated or the desired protein is separated from all other proteins. Separation of one protein from all others is usually very demanding. Separation steps usually use protein-protein differences, physicochemical properties, binding affinity, and biological activity.

Methods used to purify proteins can be roughly divided into analytical and preparative methods. The decisive factor is the amount of protein that can be purified by the method.

The aim of analytical methods is detect and identify the protein in the mixture, while the aim of preparative methods is produced a large amount of protein for other purposes, for example for industrial and biomechanical applications. Analytical purification produces a relatively small amount of protein for various research or analytical purposes, including identification, quantification and study of protein structure, post-translational modifications and functions.

Preparative methods use commercial products such as enzymes (e.g. lactases), nutritional proteins (e.g. soy protein isolate) and some biopharmaceuticals (e.g. insulin) [3].

2.2 Extraction

Depending on the source, the extracted proteins must be obtained in solution, so it is necessary to break the tissue or cells. There are several ways to achieve this: Repeated freezing and thawing, ultrasonication, high pressure homogenisation, filtration, or permeabilization with organic solvents. The method depends on how fragile or robust the protein is. Soluble proteins in the solvent may be separated from the cell membrane, e.g. centrifugation. Also, proteases that digest proteins in solution are also used. If the protein is sensitive to proteolysis, it is necessary to proceed quickly and the extract must be in a cold thermoregulatory environment, this slows the proteolysis process itself.

2.3 Precipitation

For mass purification of proteins, the first common step in protein isolation is precipitation with ammonium sulphate $(NH_4)_2SO_4$. This is accomplished by the gradual addition of ammonium sulfate to form a protein precipitate. Ammonium sulfate can be removed by dialysis. The precipitated protein will be large enough to be visible to the eye. One of the advantages of this method is that it is inexpensive and suitable for large volumes.

The purification of integral membrane proteins requires the disruption of the cell membrane to isolate one particular protein from the others in the same membrane space. Sometimes the membrane fraction can be isolated first, for example, it is necessary to isolate the mitochondria from the cells before purifying the protein found in the mitochondrial membrane. A detergent such as sodium dodecyl sulfate (SDS) may be used to dissolve cell membranes. However, because SDS causes denaturation, it is better to use milder means, such as Triton X-100, to preserve native conformation of proteins.

2.4 Centrifugation

Centrifugation is a biomechanical process that uses centrifugal forces to separate particles of different masses or densities dispersed in the liquid.When the tube, with a mixture of proteins or other particles, rotates at high speed, the particle suspension is rotated in a centrifuge. On the bottom of the tube, pellets are formed where heaviest particles are. The incompatible particles remain mostly in the liquid, in the supernatant, and can be removed from the pellet by pipetting. The spin speed is determined by the angular acceleration applied to the sample. This is usually measured as compared to gravitational acceleration [2].

2.5 Solubilization

The solubility (influence of ionic strength) of proteins at low ionic strengths increases with the salt concentration - salting in. This is because, at low ionic strengths, attractive interactions exist between protein molecules. Through back-charged portions of these molecules, their solubility is reduced. By increasing the salt concentration, the magnitude of these interactions decreases, thereby increasing the solubility. By increasing the salt concentration, the magnitude of these interactions decreases, thereby increasing the solubility. At very high ionic strengths, the charges on the protein molecules are shielded ⇒ there are only very weak electrostatic interactions between the protein molecules leading to low solubility. This phenomenon is called salting out. Salting out is one of the most important processes for the isolation and purification of proteins.

3 Izoelectric focusing

The first step of 2D electrophoresis (SDS-PAGE) is isoelectric focusing (IEF), Figure 3.

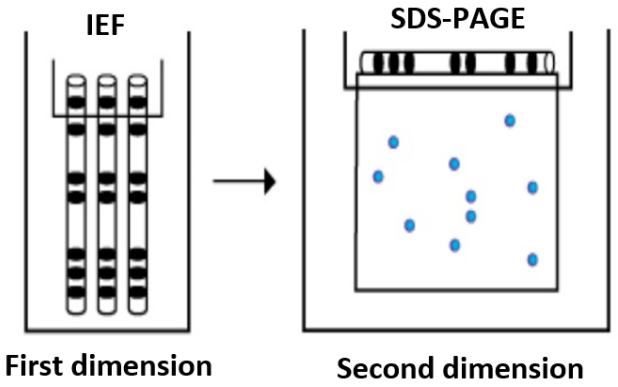

Figure 3 Separation of proteins ***Chyba! Nenašiel sa žiaden zdroj odkazov.***

Division by this method is after application of voltage, by migration of proteins in a variable pH environment. In a basic environment, the proteins have a negative charge and are therefore traveling to the anode. In the acid environment, on the contrary, the total charge is positive and they are traveling to the cathode. At a pH equal to the isoelectric point, pI, the molecule is neutral and focuses at this point because at this point the total charge of the protein is zeroed. The isoelectric point (pI) is the pH value of the solution at which the molecule, resp. the set of all molecules originating from the starting molecule in the solution is electroneutral, i.e. it has no electrical charge.

The protein breaks out of the isoelectric point immediately as soon as it gets the charge, and it returns back to the isoelectric point due to the tension. By means of isoelectric focusing, we can achieve protein separations. They differ in isoelectric points by only 0.01. Isoelectric focusing takes place on IPG strips, Figure 4.

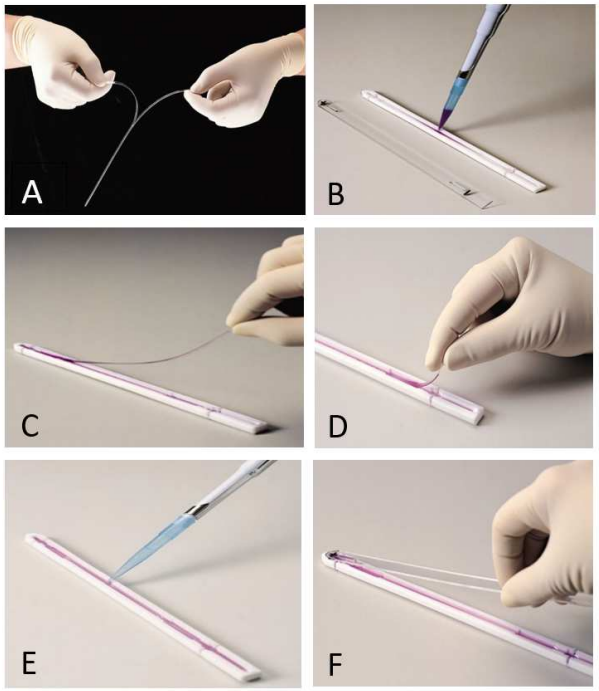

Figure 4 IPG strip: A: remove protective film, B: Apply rehydration solution to the strip, C: wet entire length of IPG strip in rehydration solution by placing IPG strip in strip holder (gel facing down), D: gently lay entire IPG strip in the strip holder, placing the end of IPG strip over cathode electrode. E: protein sample can be applied at sample application well following the rehydration step if the protein sample was not included in the rehydration solution, F: place cover on strip holder [3]

The strips are inserted into the IEF. High voltages (up to several tens of kilovolts) are used [4,5].

4 Conclusions

Different types of cells contain different proteins, so proteome of a cell will be different from another cell proteome. In addition, cells that are the result of diseases such as cancer, have a different proteome than normal cells. For this reason, understanding the "normal" proteome of a cell is critical to understanding the changes that occur as a result of the disease. This knowledge can

lead to an understanding of the molecular basis for diseases that can then be used to develop treatment strategies.

Acknowledgement
This article was supported by the state grants KEGA 069TUKE-4/2017(50%) of the Ministry of Education of the Slovak Republic and APVV-15-0356 (50%) MEDPEEK.

References

[1] www.fnspba.sk/aktuality-subory/postery/C19.ppt, 2018. (Original in Slovak)
[2] http://en.wikipedia.org/wiki/Protein_purification, 2018.
[3] https://www.slideshare.net/sindoori/iso-electro-foucing-nitu, 2018.
[4] ŽIVČÁK, J., TREBUŇOVÁ , M.: Basic principles of isoelectric focusing in biomedical engineering, Transfer inovácií, Vol. 2013, No. 28, pp. 222-224, 2013.
[5] TREBUŇOVÁ, M., LAPUTKOVÁ, G., Živčák, J.: Izoelektrická fokusácia v biomedicínskom inžinierstve, Transfer inovácií, Vol. 2013, No. 28, pp. 225-228, 2013. (Original in Slovak)

Review process
Single-blind peer review process.