JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2006, 57, Supp 7, 81–93
www.jpp.krakow.pl

A. HEROSIMCZYK[1,2], N. DEJEANS[2], T. SAYD[3], M. OŻGO[1],
W. F. SKRZYPCZAK[1], A. MAZUR[2]

PLASMA PROTEOME ANALYSIS: 2D GELS AND CHIPS

[1] Department of Animal Physiology and Cytobiology, Agricultural University, Szczecin, Poland, [2]Equipe Stress Métabolique et Micronutriments, Unité de Nutrition Humaine, INRA Clermont-Ferrand/Theix, Saint Genès, Champanelle, France, [3] Station de Recherche sur la Viande, Institut National de la Recherche Agronomique, Saint-Genès, Champanelle, France

The knowledge of concentration, modification and interaction of proteins is fundamental in determining the phenotype of living organisms. Plasma, the primary clinical specimen, contains numerous and diverse proteins. The functions of these proteins are as manifold as the diversity of the protein themselves. Many of them have been largely used for many years as biomarkers of diseases and indicators of the physiological functions. The study of plasma proteome promises to be a significant advance in various areas of biological and clinical research. Two-dimensional polyacrylamide gel electrophoresis is considered as a primary tool in separating thousand of plasma proteins. This approach enables comparing normal and diseased samples revealing differently expressed proteins. Other proteomic techniques suitable for plasma analysis such as protein microarrays are now either established or are still being improved. This article briefly reviews the application of two-dimensional electrophoresis and the current status of technical aspects for plasma proteome.

Key words: *Two-dimensional electrophoresis; proteomics; proteome; plasma; protein chips.*

INTRODUCTION

Proteomics is the branch of science that characterizes precisely the entire proteome by analyzing the structure and functions of all proteins present in cells or body fluids (1). The primary aim of proteomic analysis is to separate, identify and characterize proteins and understand their interactions with other proteins. Proteomic analysis can be used on tissue samples and also on body fluids such as

serum, plasma, urine, saliva, exudates… (2). Many domains of biological sciences are using now proteomics as a valuable research tool.

Plasma or serum is a very precious source for proteomic researches because it contains 60-80 mg/ml of proteins and it is also easy to obtain from patients (3 - 6). Many of these proteins are secreted and are shed from cells and tissues from all over the body (3). Several plasma proteins have been recognized from many years as valuable physiological and pathological markers. Now the progress in proteomics provides new basis for the better knowledge of plasma or serum proteins as potential biomarkers in several diseases (7 - 16). By comparing the different protein expression profiles between normal and diseased plasma samples valuable information about the process of pathogenesis can be obtained.

There are many varied technologies used in proteomics. One of the most effective and widely used methods is a combination of two-dimensional gel electrophoresis (2-DE) to separate proteins and mass spectrometry to identify and characterize the proteins (17 - 21). Other new approach for profile proteins are protein microarrays that are employed for the expression analysis of set of proteins.

PLASMA SAMPLE PREPARATION FOR 2-DE

One of the greatest challenges of proteome plasma analysis is to choose an adequate method of sample preparation. This is the most important stage in proteome analysis and it may have a great influence on the result of the experiment.

The process starts by solubilizing all proteins, and this can be achieved by use of chaotropic agents, reducing agents and detergents (22).

Urea and thiourea are widely used as chaotrope for 2D-electrophoresis. Chaotropic agents disrupt hydrogen bonds and hydrophobic interactions both between and within proteins leading to their denaturation (23). It is known that thiourea is poorly soluble in water but it is more soluble in presence of urea. Urea is most frequently used at concentrations from 5 to 7 M often with 2 M thiourea (24).

The most commonly used reducing agents are dithiothereitol (DTT) or tributylophosphine (TBP). The main role of these two agents depends on cleaving disulfide bond crosslinks between and within proteins (25).

Detergents are used to break the hydrophobic interactions and increase solubility of proteins. Commonly these agents are used at concentrations of 1-4%. According to the detergent's charge in solution, detergents are classified into 4 categories: mainly anionic (e.g. SDS), cationic, neutral (e.g. Triton X-100, dodecyl maltoside) and zwitterionic (e.g. CHAPS or CHAPSO). The fact that neutral and zwitterionic detergents are most compatible with isoelectrofocusing separation makes them the most commonly applied agents for sample preparation for 2-DE (26).

It is known that 10,000 different proteins may exist in human plasma. Most of them are presented at very low abundance (3, 4). The basic problem in proteomic analysis of plasma is to find low molecular weight proteins (LMW) in the presence of high abundance proteins. The most abundant proteins are albumin (HSA) and immunoglobulin G (IgG). Albumin constitutes 57-71% and immunoglobulin G constitutes 8-26% of all plasma proteins (4, 27, 28). During the gel analysis high concentration of these two proteins masks the presence of proteins which may have a similar isoelectric point or similar molecular weight. Therefore HSA and IgG should be depleted from plasma before the remaining proteins, which are present in lower concentration can be detected. Many varied approaches have been developed to remove abundant proteins from plasma and serum. Human serum albumin can be removed by affinity columns such as Cibacron- Blue or specific antibodies (17, 27). The removal of IgG may be done based on columns containing protein G, which covalently binds and selectivity removes this abundant protein. The IgG removal can be also achieved using specific antibodies (17, 27).

As result of these technical problems, new approaches for clearing albumin and immunoglobulin G from human plasma samples have been developed.

ProtoClear™ is a polypeptide affinity matrix designed to bind highly abundant proteins without removing proteins in addition to albumin (28). The authors compared the amount of human serum albumin before and after treatment by ProtoClear™ and the results indicate that percent removal of HSA were 98% and percent of removal IgG were 97%. The results of binding high abundant proteins by ProtoClear™ were very similar to the results achieved by Cibracon Blue chromatography. However, the authors pointed out that in spite of the fact that the two techniques removed similar amounts of HSA and IgG the Cibracon blue removed many proteins in addition to albumin (28).

The other approach, which allows the binding of high molecular proteins from human plasma and to enrich specific proteins of interest, is the simple affinity spin tube filter. The affinity spin tube filter contains antibodies against high-abundant proteins or against specific proteins of interest combined with protein G (29).

Other methods based on centrifugal ultrafiltration are less successful than the methods described above because they do not completely remove albumins and immunoglobulins G from plasma samples (27, 29, 30). Centrifugal ultrafiltration membranes appear to be more useful to concentrate proteins (30).

Immunodepletion represents a new strategy for depleting high abundance proteins from plasma. For example ProteoPrep® 20 Plasma Immunodepletion Kit-PROT-20 resin removes 99% of 20 major plasma or serum proteins. The biggest advantage of this method is the ability to have a greater loading capacity and the fact that the resin can be reused many times (31).

These techniques of depletion of major proteins from the plasma are currently in development. This could undoubtedly contribute to a better assessment of plasma proteome and especially low abundant proteins.

TWO-DIMENSIONAL ELECTROPHORESIS IMMOBILIZED pH GRADIENT GEL (IPG) SYSTEM

Two-dimensional gel electrophoresis became the fundamental technique for study of protein expression. This approach is considered as the most powerful in separating a complex of proteins such as those present in cells, tissues or body fluids (32). First the proteins are separated in the first dimension by isoelectric focusing (IEF) and then by molecular weight (MW) in a second dimension using electrophoresis in gel containing sodium dodecyl sulfate (SDS-PAGE) (33).

Differences in isoelectric point (pI) of proteins are the basis of separation by IEF. The pI is defined as pH at which a protein will not migrate in an electric field. For every protein there is a specific pH at which its net charge is zero, this is its pI. In early studies carrier ampholytes were used to establish pH gradient for isoelectric focusing. However, the ampholyte technique has many drawbacks like drift during the isoelectric focusing and difficulty in achieving reproducibility due to variations in different batches of ampholytes (1). The introduction of immobilized pH gradient (IPG) for isoelectric focusing has greatly improved the gradient stability and reproducibility and also allows for high protein loads. The process of forming IPGs is based on principal that pH gradient is generated by buffering and titrant groups which are co-polymerized with acrylamide matrix (24). The most important advantage of IPGs is that the immobilized chemicals cannot migrate in the electric field thus the problem with drift during isoelectric focusing is eliminated (24). Immobilized pH gradient strips allow the generation of pH gradients of various lengths (7 cm, 13 cm, 18 cm and 24 cm) and any pH range (broad, narrow, ultra-narrow) between pH 3 and 12.

PROTEIN DETECTION METHODS

After the separation by two-dimensional electrophoresis the proteins need to be visualized. The visualization can be achieved by protein staining methods used after running the gels or protein labelling techniques used before electrophoresis (34). Coomassie brilliant blue staining is easy to perform but it shows detection limits (100 ng protein) and as a consequence, it allows only detection of major components in protein samples (34, 35). Silver staining is about 100 times more sensitive than Coomassie brilliant blue and thus can detect more of the low-abundance proteins (1, 34). *Figure 1* presents a picture of 2D gel of human serum proteins detected by staining with silver.

However this staining technique has some drawbacks like poor dynamic range, low reproducibility and also tendency for negative staining of certain spots (35). The introduction of fluorescent dyes offers an alternative to silver staining. SYPRO Orange, SYPRO Red protein gel staining and also Cy2, Cy3 and Cy5 have equivalent sensitivities to silver but have wider dynamic range and thus are more effective for staining 2D-gels (1). The development of fluorescent detection

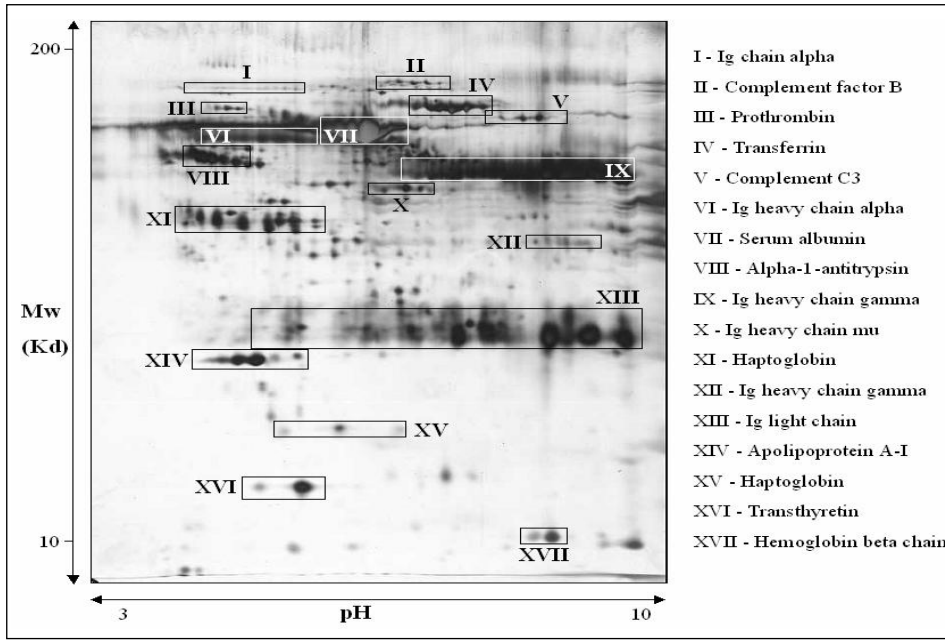

Fig. 1. Image of a 2D gel of human serum proteins after analysis using PDQuest software. First dimension: wide-range immobilized pH gradient (pH from 3 to 12); second dimension: SDS-PAGE (14% of acrylamide and bisacrylamide concentration). The proteins were detected by staining with silver (author's figure).

is in progress and will undoubtedly provide a sensitive means for the detection of proteins on the 2D gels.

COMPUTER SOFTWARES FOR ANALYSIS OF 2D IMAGES

Gel-based techniques offer the possibility for the quantitative comparison of the proteins between samples (1). Usually images of gels are captured by an image scanner or fluorescent scanner and are subsequently subjected for analysis by computer software. There are many various computer software programs suitable for analysis of 2D images including: ImageMaster (Amersham Biosciences), PDQuest (BioRad) (*Fig. 1*), Melanie (Geneva Bioinformatics). Marengo *et al.* (36) recently published a review which describes the available computer software for analysis of 2D images. These software tools are required to detect all the spots on the gel image, which are then manually edited and matched to its corresponding spot in other gels. After matching, 2-D gel patterns of similar experiments are compared with 2-D patterns of another experiment (e.g. comparing protein expression profiles between normal and diseased samples) (35). Only the proteins of interest (altered/ differently expressed) are selected for analysis by mass spectrometry.

MASS SPECTROMETRY (MS)

In general 2-DE provides useful determination of protein's pI and molecular weight but currently this information alone is insufficient to identify the proteins. Accordingly, mass spectrometry (MS) is used for protein identification after the proteins are separated by 2-DE. MS can provide protein structural information such as peptide masses, amino acid sequences or types of protein modification (37).

Two gentle ionization methods such as matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) have become the methods that enable the analysis of proteins and peptides (for review (21)). To carry out the analysis in a mass spectrometer a particle needs to be charged before it can be manipulated in an electric field. Both in electrospray ionization and in matrix-assisted laser desorption ionization a charge is transferred to the molecules (peptides) to enable it to be mass analyzed. Regardless whether ionization occurs by ESI or by MALDI, the charged peptide ions are separated by a mass analyzer. The separation is based on the ratio of their mass to charge (m/z), and then the number of ions of a given m/z value are counted by the detector (38).

One of the most popular protein identification strategies for the identification of proteins isolated with 2-DE is peptide mass fingerprinting. In this method proteins excised from gels are digested with proteolytic enzymes to produce peptide fragments specific to each protein. The mass measurement of created peptide fragments are often analyzed by Matrix–assisted laser desorption/ionization–time of–flight (MALDI-TOF) to obtain peptide fingerprints, which allow the identification of proteins through matching to a database (37).

The second method which enables the identification of the amino acid sequence of unknown peptides is peptide sequencing by tandem mass spectrometry. In this technique peptides are first selected from the entire peptide mixture in the first mass spectrometer and subsequently fragmented by collision with an inert gas. Obtained in that way, peptide fragments are separated in the second mass spectrometry (the second part of tandem mass spectrometer), producing partial or complete sequence of a peptide (so called 'peptide sequence tag'). Data obtained in this process permit further identification of proteins from databases (for review 39).

PROTEIN MICROARRAYS

Another new approach for profiling proteins is protein microarrays, which are employed to assess differences in their expression. The idea of a protein chip was inspired by DNA microarrays, which allow for monitoring the expression of thousands of genes in a single experiment (40, 41). Generally, there are two formats of protein microarrays: the protein chip, which contains large numbers of proteins and the antibody chip, which consists of capture molecules, immobilised in various surfaces such as plain glass, porous acrylamide gel, membranes and

microwells (42). The former can be created from any type of protein and therefore is more useful. This type of microarray can be used to screen large numbers of proteins for biochemical activities, protein-protein, protein-lipid, protein-nucleic acid and small molecule interactions (43). The antibody microarrays are designed to detect specific components of complex samples. They are made by printing microscopic spots of specific antibodies onto surfaces. Next the biological sample such as plasma is localized on the chip surface let the antigens bind to their specific antibodies. There are two methods of detecting protein expression with antibody arrays. In the first detection of the bound antigen is achieved using fluorescently tagged or radioactively labelled proteins (21). The second method is based on sandwich immunoassays with secondary antibodies against each antigen of interest. This type of protein array requires two antibodies for each protein to be detected. The first antibody binds the protein and subsequently the second (labelled) antibody binds to a different region of the protein than the capture antibody. The protein – antigen complex is detected for example by using streptavidin conjugated such as horseradish peroxidase (HRP) (40, 44). This method is known to be successful for monitoring protein expression levels. For example, as presented in the *Fig. 2*, multiple cytokine expression can be measured simultaneously by protein chip technology.

	a	b	c	d	e	f	g	h
1	Pos	Pos	Neg	Neg	GCSF	GM-CSF	GRO	GRO-alpha
2	Pos	Pos	Neg	Neg	GCSF	GM-CSF	GRO	GRO-alpha
3	IL-1 alpha	IL-2	IL-3	IL-5	IL-6	IL-7	IL-8	IL-10
4	IL-1 alpha	IL-2	IL-3	IL-5	IL-6	IL-7	IL-8	IL-10
5	IL-13	IL-15	IFN-gamma	MCP-1	MCP-2	MCP-3	MIG	RANTES
6	IL-13	IL-15	IFN-gamma	MCP-1	MCP-2	MCP-3	MIG	RANTES
7	TGF-beta 1	TNF-a	TNF-beta	Blank	Blank	Blank	Blank	Pos
8	TGF-beta 2	TNF-a	TNF-beta	Blank	Blank	Blank	Blank	Pos

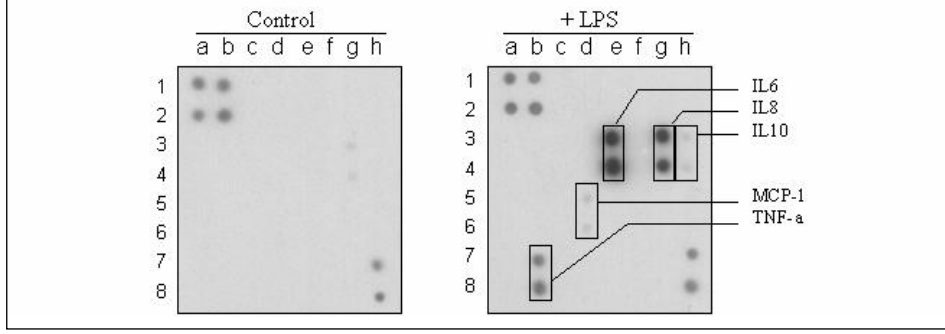

Fig. 2. Example of protein chip analysis of the plasma. Whole human blood was exposed for 6 hrs in 37°C to the lipopolysaccharide (LPS) (10ng/ml *E. coli* serotype 055:B5, Sigma). Cytokine production was analyzed by using Ray Bio ® Human Cytokine Antibody Array (RayBiotech, Inc.). The grid of the used chip is presented at the top of this figure (Pos – positive reaction, Neg – negative reaction) (author's figure).

The biggest problem in antibody array technology is the source of capture molecules that can be used on antibody chips. Currently monoclonal antibodies (mAb) are the most common used capture agents. However, the labour intensive production and the high cost are the main obstacles to create antibody microarrays of great diversities (40, 42). To overcome this problem many alternative methods have been developed. The phage - display technique is a new approach, which holds a great promise in this field. Another strategy for gaining capture agents is peptide aptamers (45). Aptamers are single-stranded oligonucleotides that range in size between 30 and 70 nucleotides in length. Because of the fact that aptamers have the capacity to fold into three-dimensional structures they can bind to a variety of molecules including proteins, peptides, enzymes and antibodies (46, 47). These short oligonucleotides are obtained with the aid of technique called the systematic evolution of ligands by exponential enrichment (SELEX). This technology allows isolation of nucleic acids with expected biochemical properties from DNA libraries. After several cycles of selection and amplification, the population of oligonucleotides is enriched with those which bind with high affinity to the specific molecular targets for example proteins (48). The fact that aptamers can be easily produced by chemical synthesis and that they are robust and stable make them a novel molecular tool for use in assays or as diagnostic and therapeutic agents (48).

OTHER METHODS FOR PROTEINS PROFILING: MULTIDIMENSIONAL PROTEIN IDENTIFICATION TECHNOLOGY (MUDPIT)

Another alternative strategy for proteins separation is multidimensional protein identification technology (MudPIT). In MudPIT method proteins or peptides are digested with one or more enzymes such as trypsine. The resulting peptide mixture is then separated by two-dimensional chromatography using strong cation exchange as a primary separation technique and reverse-phase as a secondary technique. Separated peptides are then identified using tandem mass spectrometry (MS/MS). The main advantage of this approach is that it allows the separation and identification of proteins without the need for labelling before or after the separation (49).

APPLICATION OF PROTEOMICS

The applications of proteomics are very broad and they include the examination of protein structure and functions, and also the study of protein expression and modification under a given biological condition (37). According to this proteomics can be classified into three types. The first type is protein expression proteomics, which examines differences between protein expressions in tissues, body fluids or cells. The second type is structural proteomics. The main goal of this approach is to map out the structure of protein complexes. Functional proteomics is the third type, and it examines proteins that demonstrate a specific

biological function and it also allows for the monitoring of their protein-protein interactions and posttranslational modifications (37).

Plasma continuously perfuses all the tissues so it is believed that within this fluid lies very important information about many processes that are taking place in the human body. Identification of the plasma proteins enable the discovery of biomarkers, which can be detected and measured for disease diagnosis.

Much research has shown that plasma or serum proteins are classified as potential biomarkers in cancers such as hepatocellular cancer (12, 13), ovarian cancer (10, 15) prostate cancer (9) and breast cancer (11, 14). Neoplastic diseases do not show clinical symptoms until the disease is in advanced stages. According to this, substantial efforts were made by investigators to find tumour-associated biomarkers that enable early detection of the pathological state. For example Rui *et al*. (11) used 2-DE to find differences between normal human serum and breast cancer serum. By comparing the serological 2-D maps, the authors found two small molecular weight proteins that are potential biomarkers for breast cancer. These proteins were identified as 14-3-3 sigma protein and HSP 27 protein.

Currently the only marker available for ovarian cancer diagnosis is cancer antigen 125 (CA125). Unfortunately only 50 to 60% of women with early stage of disease reveal plasma elevations of CA125. Thus, attempts have been made to discover additional markers to identify early stage disease. Petricoin *et al.* (10) used Surface-enhanced laser desorption/ionization time of flight (SELDI-TOF) technique to profile serum samples of patients with different stages of ovarian cancer together with normal controls. In the first phase mass spectra from serum samples of 50 patients diagnosed with ovarian cancer and 50 controls were compared. In this way the authors obtained an algorithm which allowed one to distinguish the normal from diseased samples. Using these algorithm 117 masked patient samples were analyzed and the authors achieved sensitivity of 100% and specificity of 95% in discriminating ovarian cancer from non-cancer. Also Ye *et al.* (15) has identified haptoglobin α as novel ovarian cancer serum biomarkers with the aid of Surface-enhanced laser desorption/ionization mass spectrometry (SELDI-MS) and liquid chromatography/tandem mass spectrometry (LC-MS/MS) technologies.

The short supply of specific biomarkers for hepatocellular carcinoma (HCC) prompted investigators to discover new disease-associated proteins by means of proteomics (12, 13). Steel *et al.* (13) used two-dimensional electrophoresis to resolve and compare proteins presented in serum samples obtained from healthy patients and diagnosed patients with HCC. The authors identified two proteins namely C3 component and ApoA1; the levels are low in patients with diagnosis of HCC and they might be potential biomarkers for this disease.

Proteomic technologies have been also used to study hepatitis B virus (HVB) inflammation. He *et al*. (7) compared normal serum samples with HBV serum samples and they found seven proteins that might be considered as HBV biomarkers. These proteins were identified to be haptoglobin β and $\alpha 2$ chain,

apolipoprotein A-I and A-IV, α1-antitrypsin, transthyretin and DNA topoisomerase IIβ (7). Biomarker discovery by proteomics for other diseases such as Alzheimer disease (AD) is limited but holds great promise and encouragement for further investigations (8). Recent data also are available for the proteome of patients with cardiovascular diseases (16).

Proteomics is a suitable and powerful approach, which enables characterisation of proteins involved in pathological process and thus it is a technique of choice for drug discovery (50). The role of proteomics in drug development includes target identification and insight into mechanisms of drug action or toxicity (50). Proteomics also represents a new generation of toxicology methodology based on the search for early biomarkers that can predict toxic effects and could provide new early and highly specific biomarkers for the action of toxics (51).

FUTURE PROSPECTS IN PROTEOMICS

The knowledge of the plasma proteome is a high importance challenge for both scientific and clinical issues. Its development involves many technological improvements in sample processing, techniques of protein separation and identification. In particular improvements are still needed in raising the capacity for analyzing large size of samples and refining the resolution for separating low abundant proteins. In parallel the development of highly performant informatic tools will contribute to the more precise analyses and generation of precious databases. Several initiatives have been launched to organize proteome research. One of the most important is the Human Proteome Organization (HUPO). In the recent special issue of Proteomics (52), several papers from this organization present in an extended manner the specific points related to the technical development and possible applications of the plasma proteome.

Acknowledgments: The authors thank Christophe Chambon for comments and reading the text.

REFERENCES

1. Honore B, Ostergaard M, Vorum H. Functional genomics studied by proteomics. *Bioessays* 2004; 26: 815-822.
2. Kennedy S. Proteomic profiling from human samples: the body fluid alternative. *Toxicol Lett* 2001; 120: 379-384.
3. Adkins JN, Varnum SM, Auberry KJ, et al. Toward a human blood serum proteome: analysis by multidimensional separation coupled with mass spectrometry. *Mol Cell Proteomics* 2002; 1: 947-955.
4. Merrel K, Southwick K, Graves SW, Esplin MS, Levis NE, Thulin CD. Analysis of low-abundance low molecular-weight-serum proteins using mass spectrometry. *J Biomol Tech* 2004; 15: 238-248.
5. Tirumalai RS, Chan KC, Prieto DA, Issaq HJ, Conrads TP, Veenstra TD. Characterization of low molecular weight human serum proteome. *Mol Cell Proteomics* 2003; 2: 1096-1103.

6. Putnam FW. Ed. The plasma proteins. Academic Press New York , 1975; p. 481.
7. He QY, Lau GKK, Zhou Y, et al. Serum biomarkers of hepatitis B virus infected liver inflammation: a proteomic study. *Proteomics* 2003; 3: 666-674.
8. Irizarry MC. Biomarkers of Alzheimer disease in plasma. *NeuroRx* 2004; 1: 226-234.
9. Petricoin III EF, Ornstein DK, Paweletz CP, et al. Serum proteomic patterns for detection of prostate cancer. *J Natl Cancer Inst* 2002; 94: 1576-1578.
10. Petricoin EF III, Ardekani AM, Hitt BA, et al. Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* 2002; 359: 572-577.
11. Rui Z, Jian-Guo J, Yuan-Peng T, Bing-Gen R. Use of serological methods to find biomarkers associated with breast cancer. *Proteomics* 2003; 3: 433-439.
12. Seow TK, Liang RC, Leow CK, Chung MC. Hepatocellular carcinoma : from beside to proteomics. *Proteomics* 2001; 1: 1249-1263.
13. Steel LF, Shumpert D, Trotter M, et al. A strategy for the comparative analysis of serum proteomes for the discovery of biomarkers for hepatocellular carcinoma. *Proteomics* 2003; 3: 601-609.
14. Somiari RI, Somiari S, Russel S, Shriver CD. Proteomics of breast carcinoma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005; 815: 215-225.
15. Ye B, Cramer DW, Skates SJ, et al. Haptoglobin-α subnit as potential serum biomarker in ovarian cancer: identification and characterisation using proteomic profiling and mass spectrometry. *Clin Cancer Res* 2003; 9: 2904-2911.
16. Anderson L. Candidate-based proteomics in the search for biomarkers of cardiovascular disease. *J Physiol* 2005; 563: 23-60.
17. Bjorhall K, Miliotis T, Davidsson P. Comparision of different depletion strategies for improved resolution in proteomic analysis of human serum samples. *Proteomics* 2004; 5: 307-317.
18. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitive analysis of complex protein mixtures using isotope-coded affinity tags. *Nat Biotechnol* 1999; 17: 994-999.
19. Lauber WM, Caroll JA, Dufield DR, Kiesel JR, Radabaugh MR, Malone JP. Mass spectrometry compatibility of two-dimensional gel protein stains. *Electrophoresis* 2001; 22: 906-918.
20. Yates JR III. Mass spectrometry and the age of the proteome. *J Mass Spectrom* 1998; 33: 1-19.
21. Zhu H, Bilgin M, Snyder M. Proteomics. *Annu Rev Biochem* 2003; 72: 783-812.
22. Link AJ. 2-D Proteome Analysis Protocols. *Methods Mol Biol* 1999; 112.
23. Rabilloud T. Use of thiourea to increase the solubility of membrane proteins in two-dimensional electrophoresis. *Electrophoresis* 1998; 19: 758-760.
24. Molloy MP. Two dimensional ectrophoresis of membrane proteins using immobilized pH gradients. *Anal Biochem* 2000; 280: 1-10.
25. Herbert BR, Molloy MP, Gooley AA, Walsh BJ, Bryson WG, Williams KL. Improved protein solubility in two-dimensional electrophoresis using tributyl phosphine as reducing agent. *Electrophoresis* 1998; 19: 845-851.
26. Berkelman T, Brubacher MG, Chang H. Important factors influencing protein solubility for 2-D electrophoresis. Bio-Rad Laboratories 2004; Inc., Hercules, CA 94547
27. Govorughina NI, Keizer-Gunnik A, van der Zee AG, de Jong S, de Bruijn HW, Bischoff R. Sample preparation of human serum for the analysis of tumor markers. Comparison of different approaches for albumin and gamma-globulin depletion. *J Chromatogr A* 2003; 1009: 171-178.
28. Lollo BA, Harvey S, Liao J, et al. Improved two-dimensional gel electrophoresis representation of serum proteins by using ProtoClear™. *Electrophoresis* 1999; 20: 249-259.
29. Wang YY, Cheng P, Chan DW. A simple affinity spin tube filter for removing high-abundant common proteins or enriching low-abundant biomarkers for serum proteomic analysis. *Proteomics* 2003; 3: 243-248.

30. Georgiou HM, Rice GE, Baker MS. Proteomic analysis of human plasma: failure of centrifugal ultrafiltration to remove albumin and other high molecular weight proteins. *Proteomics* 2001; 1: 1503-1506.
31. Schuchard MD, Melm CD, Crawford AS, et al. Immunoaffinity depletion of 20 high abundance human plasma proteins. Sigma-aldrich.com
32. Gromov PS, Ostergaard M, Gromova I, Celis JE. Human proteomic databases: a powerful resource for functional genomics in health and diesease. *Prog Biophys Mol Biol* 2002; 80: 3-22.
33. Sellers TA, Yates JR. Review of proteomics with applications to genetic epidemiology. *Genet Epidemiol* 2003; 24: 83-98.
34. Chambers G, Lawrie L, Cash P, Murray GI. Proteomics: a new approach to the study of disease. *J Pathol* 2000; 192: 280-288.
35. Görg A. Advances in 2D gel techniques. Proteomics: A Trends Guide. Elsevier Science 2000; 3-6.
36. Marengo E, Robotti E, Antonucci F, Cecconi D, Campostrini N, Righetti G. Numerical approaches for quantitative analysis of two-dimensional maps: a review of commercial software and home-made systems. *Proteomics* 2005; 5: 654-666.
37. He QY, Chiu JF. Proteomics in biomarker discovery and drug development. *J Cell Biochem* 2003; 89: 868-886.
38. Patterson SD. Proteomics: beginning to realize its promise. *Arthritis Rheum* 2004; 50: 3741-3744.
39. Mann M, Hendrickson RC, Pandey A. Analysis of proteins and proteomes by mass spectrometry. *Annu Rev Biochem* 2001; 70: 437-473.
40. Sydor JR, Nock S. Protein expression profiling arrays: tools for the multiplexed high-throughput analysis of proteins. *Proteome Sci* 2003; 1: 3.
41. Wilson DS, Nock S. Recent developments in protein microarray technology. *Angew Chem Int Ed Eng* 2003; 42: 494-500.
42. Glokler J, Angenendt P. Protein and antibody microarray technology. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003; 797: 229-240.
43. Schweitzer B, Predki P, Snyder M. Microarrays to characterize protein interactions on a whole-proteome scale. *Proteomics* 2003; 3: 2190-2199.
44. Baary R, Solovier M. Quantitative protein profiling using antibody arrays. *Proteomics* 2004; 4: 3717-3126.
45. Templin MF, Stoll D, Schrenk M, Traub PC, Vohringer CF, Joos TO. Protein microarray technology. *Drug Discov Today* 2002; 7: 815-822.
46. Rimmele M. Nucleic acid aptamers as tools and drugs: recent developments. *Chembiochem* 2003; 4: 963-971.
47. Missailidis S, Thomaidou D, Borbas KE, Price MR. Selection of aptamers with high affinity and high specifity against C595, an anti-MUC1 IgG3 monoclonal antibody, for antibody targeting. *J Immunol Methods* 2005; 296: 45-62.
48. Cerchia L, Duconge F, Pestourie C, et al. Neutralizing aptamers from whole-cell SELEX inhibit the RET receptor tyrosine kinase. *PloS Biol* 2005; 3: e123.
49. Delahunty C, Yates III JR. Protein identification using 2D-LC-MS/MS. *Methods* 2005; 35: 248-255.
50. Walgren JL, Thompson DC. Application of proteomic technologies in the drug development process. *Toxicol Lett* 2004; 149: 377-385.
51. Fella K, Gluckmann M, Hellman J, Karas M, Kramer PJ, Kroger M. Use of two-dimensional gel electrophoresis in predictive toxicology: Identification of potential early protein biomarkers in chemically induced hepatocarcinogenesis. *Proteomics* 2005; 5: 1914-1927.

52. Omenn GS. Ed.: Exploring the human plasma proteome. The HUPO plasma proteome project (PPP). *Proteomics* 2005; 5: 3223-3550.

Received: September 14, 2006
Accepted: October 10, 2006

Author's address: Andrzej Mazur, Ph.D., Centre de Recherche en Nutrition Humaine d'Auvergne, Unité de Nutrition Humaine, INRA, Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France; e-mail: mazur@clermont.inra.fr