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Journal of Chromatography B, 815 (2005) 179–189
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Proteomics analysis of human cerebrospinal fluid

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Available online 29 July 2004

Abstract

Cerebrospinal fluid (CSF) is secreted from several different central nervous system (CNS) structures, and any changes in the CSF composition will accurately reflect pathological processes. Proteomics offers a comprehensive bird's eye view to analyze CSF proteins at a systems level. This paper reviews the variety of analytical methods that have been used for proteomics analysis of CSF, including sample preparation, two-dimensional liquid and gel electrophoresis, mass spectrometry, bioinformatics, and non-gel methods. The differentially expressed CSF proteins that have been identified by proteomics methods are discussed.

Keywords: Reviews; Proteomics; Cerebrospinal fluid

Contents

Abbreviations: 2DGE, two-dimensional gel electrophoresis; $[M + H]^+$, protonated molecule ion; CSF, cerebrospinal fluid; DEP, differentially expressed protein; ESI, electrospray ionization; FTICR, Fourier-transform ion-cyclotron resonance; IT, ion trap; LC, liquid chromatography; LP-IEF, liquid-phase isoelectric focusing; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; PMF, peptide mass fingerprinting; PSD, post-source decay; Q, quadrupole; SPE, solid-phase extraction; TOF, time-of-flight

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doi:10.1016/j.jchromb.2004.06.044

1. Introduction

Cerebrospinal fluid (CSF) circulates within the ventricles of the brain, and surrounds the brain and spinal column. CSF is secreted from several different central nervous system (CNS) structures; in particular, from the ventricular chorioid plexus [1]. The total volume of CSF in the human ventricular system is ca. 125 ml [2], ca. 500 ml of CSF is produced per day, and some CSF seeps down around the spinal cord. CSF contains small molecules, salts, peptides, proteins, enzymes, etc. that play critical roles in many physiological processes. Changes (concentration; modification of proteins and peptides) in CSF compositions accurately reflect pathological processes in the CNS, and CSF offers a unique window to study CNS disorders.

The proteome has been described as all of the proteins that are produced at a given time from the genome in a cell, tissue, or fluid [3,4]. Proteomics combines two-dimensional gel electrophoresis (2DGE), high-sensitivity mass spectrometry (MS), and continuously developing bioinformatics algorithms and databases. Proteomics offers a comprehensive, bird's eye view to analyze, at a systems level, all of the proteins that might result from, or contribute to, each different CNS disorder. The CSF proteome could provide unique biomarkers for the early-stage diagnosis or the staging of a neuronal disease, offer potential insights into the biochemical characterization of affected neuronal populations, and clarify the basic molecular basis of CNS pathologies.

2. Sample-preparation methods for the two-dimensional gel electrophoresis (2DGE) analysis of CSF

CSF contains a high salt concentration (>150 mmol/L) and a low protein concentration (the CSF/serum ratio of protein concentration is 4×10^{-3}, ca. 200–700 μg protein/mL). A high level of salt interferes with the electrophoretic separation of proteins because of the high electrical current that is carried by the salt load and that reduces the efficiency of the 2DGE. For an analytical 2D gel, generally, ca. 70 μg protein, corresponding to ca. 100–350 μl of a CSF sample, is loaded. After sample dissolution, the salt concentration in the rehydration buffer should be <10 mmol/L [5]. Therefore, it is necessary to remove >80% salt of a CSF sample prior to 2DGE to remove that interference, and to preferentially enrich the proteins.

2.1. Four different sample preparation methods—ultrafiltration, dialysis, protein precipitation, and Bio-Spin column

Four different desalting methods ultrafiltration, dialysis, protein precipitation, and Bio-Spin column have been reported. (1) *Ultrafiltration*: ultrafitration was performed with an Ultrafree-MC centrifugal filter (cut-off mass: 5 kDa). The salt and other impurities (<5 kDa) passed through the filter (ultrafiltrate), and the peptides and proteins (>5 kDa) were retained (the retentate) and were concentrated [6]. (2) *Dialysis*: overnight dialysis was performed with a membrane (cut-off mass: 3.5 kDa), and proteins were either precipitated with acetone [7] or were concentrated with a vacuum [8]. (3) *Protein precipitation*: a CSF sample was incubated with ice-cold acetone [6,9], TCA in acetone [9], or ethanol [10]. After centrifugation, proteins were precipitated. The pellet was washed twice, and was used for 2DGE. (4) *Bio-Spin column*: a CSF sample was loaded onto a polyacrylamide micro-column (cut-off mass: 6 kDa). After centrifugation (1000 × g; 4 min), salts and other impurities (<6 kDa) were bound to the column; peptides and proteins (>6 kDa) were eluted the column [8,10].

2.2. Comparison of different sample-preparation methods

As the resolution of the 2D gel and the protein recovery are two critical evaluation factors for any sample-preparation method, it is important to preferentially enrich the proteins and to remove any salts and other impurities. Table 1 compares the protein recoveries of those four different sample-preparation methods. Protein adsorption on the filter is the main source of protein loss during ultrafiltration,. Protein loss during ultrafiltration is less than that for dialysis [7]. The different protein recoveries [6,9] probably reflect the different protein-measurement methods. Protein precipitation with TCA in acetone leads to a relatively low protein recovery, whereas the highest recovery was obtained with the Bio-Spin column salt-removal method.

Table 1
Comparison of protein recoveries of different sample-preparation methods

Method	Recovery (%)	Ref.
Ultrafiltration	70	Sickmann et al. [6]
Dialysis	40–60	Hammack et al. [7]
Proteinprecipitation		
Acetone	94	Yuan et al. [9]
	40–50	Sickmann et al. [6]
TCA in acetone	23	Yuan et al. [9]
Ethanol	–	Choe et al. [10,32]
Bio-Spin column	91	Yuan et al. [9]
	99	Terry and Desiderio [11]

(–) No data provided.

The high level of resolution that is routinely obtained on a 2D gel facilitates the image analysis, and improves the confidence level for accurately determining differential spots in comparative proteomics. Even though the protein recovery was high with acetone–protein precipitation method, horizontal and vertical streaks on the gel caused a high level of background. As image-analysis software could not readily distinguish protein spots from that background, the number of spots decreased. The 2D gel was clearer when the CSF sample was pre-fractionated with a Bio-Spin column, and more spots were detected [9]. Therefore, salt-removal with a Bio-Spin column was preferred because of its high level of protein recovery and the improved gel resolution.

3. Prefractionation of CSF

Some low-abundance CSF proteins are disease-associated, but they are difficult to characterize due to several factors: a low total-protein concentration (the CSF/serum ratio of protein concentration is ca. 4×10^{-3}), a high amount of albumin and immunoglobin proteins (albumin > 50% and immunoblobins > 15% of the total protein content in human CSF), and a wide dynamic range of protein concentration. Precipitation of high-abundance proteins usually occurs during isoelectric focusing (IEF), and an increased number of horizontal and vertical streaks occur on a 2D gel if too much sample is loaded to visualize the low-abundance proteins. Those low-abundance proteins are not detected either due to their low levels or to the interference on the gel from neighboring high-abundance proteins. Therefore, prefractionation of CSF is needed to preferentially enrich low-abundance proteins.

3.1. Affinity removal of albumin and immunoglobins

Raymackers et al. [12] took advantage of the affinity interactions between albumin and Cibacron Blue F3G-A (Blue Sepharose 6 Fast Flow), and between immunoglobulins and protein G (Prosep-G) to deplete the high-abundance CSF proteins–albumin and immunoglobins. The experimental results indicated that most of the albumin and immunoglobins was removed, and that several new spots appeared. However, some other proteins also disappeared. It has been well-known that albumin can bind with many different proteins. Affinity purification was performed under near-physiological conditions (pH 7.0, 0.05 M Tris–HCl, 0.1 M KCl). Those proteins that remained bound to albumin could not be released, and were retained on the affinity column. That phenomenon is a major limitation of that affinity method. Another difficulty was the binding of Cibacron Blue F3G-A to several lipoproteins and enzymes [7]. At present, many commercial affinity albumin-removal kits have been designed specifically for use with serum. Therefore, because the protein concentration is much higher in serum than that in CSF, those kits and their recommended procedures require specific modifications and optimizations in order to be compatible with CSF. For example, the concentration of CSF protein with a commercial kit might be necessary prior to affinity removal of albumin.

3.2. Liquid-phase isoelectric focusing

Davidsson et al. [13] prefractionated a CSF sample with liquid-phase IEF. CSF proteins were separated, according to charge, into 20 fractions. The proteins in selected fractions were precipitated with acetone, and were separated with 2DGE. Generally, more protein spots were visualized in the 2D gel of prefractionated CSF, and the different post-translationally modified protein forms were enriched and better resolved, compared to the 2D gel of unfractionated CSF.

3.3. Solid-phase extraction (SPE)

Yuan and Desiderio [14] developed a prefractionation method that was based on the different hydrophobic properties of CSF proteins. A CSF sample was prefractionated into three fractions with a SPE cartridge prior to 2DGE. Some of the high-abundance CSF proteins were removed from two of those three fractions; some low-abundance CSF proteins were preferentially enriched in those two fractions, and 2D electrophoretic resolution was improved to separate those low-abundance proteins from any of the neighboring high-abundance proteins. Many low-abundance proteins were detected on the 2D gels of prefractionated CSF, were the first to be MS-characterized, and were annotated on the 2D gel map of CSF.

4. CSF protein mapping

Many proteomics analyses of CSF have focused on protein mapping. Proteins were separated with 2DGE; each selected protein was digested with trypsin; the tryptic peptides were analyzed with MS; and either the peptide mass fingerprint (PMF) or the amino acid sequence data were input to the protein database to search for the matching protein.

4.1. 2DGE

At present, 2DGE is a popular method to separate a complex mixture of proteins. 2DGE [6,9,12,15,16] consists of first-dimensional gel electrophoresis—IEF to separate proteins according to isoelectric point, and second-dimensional gel electrophoresis—sodium doclecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) to separate proteins according to molecular weight. The application of a commercial immobilized pH gradient (IPG) strip has greatly improved 2DGE reproducibility. The longer (18 or 24 cm) and narrower-p*I* ranges (even 1 U/strip) strips have increased significantly the electrophoretic resolution of proteins and the sample load. Some 2DGE systems can simultaneously analyze up to 12 gels to minimize any difference between-gels that would be caused by instrumental conditions. Coomassie Blue staining and silver staining were used to visualize proteins. More protein spots were observed with silver staining because of its relatively high level of detection sensitivity. Fig. 1 contains a representative 2D gel (p*I* range: 3–10 linear) of a human lumbar CSF sample.

4.2. Preparative 2D liquid-phase electrophoresis

A preparative 2D liquid-phase electrophoresis (2D LPE) system for the pre-separation of CSF proteins has been reported [17–22]. That method is similar to 2DGE; the only difference is the liquid IEF. IEF-separated CSF proteins were collected into 20 cells, and the proteins in each cell were separated with SDS–PAGE. The advantage of that 2D-LPE method was an increased sample-loading amount for the 2D gel and less discrimination of membrane proteins, which have lower transfer efficiency from the first to the second dimension in 2DGE. The disadvantage of the 2D-LPE method is the decreased level of LPE resolution for protein separation.

4.3. MS-identification of 2DGE-separated proteins

The recent developments in biological MS have commensurately improved research capabilities in the life sciences. The 2002 Nobel Prize for chemistry was awarded to the inventors of soft-ionization methods in MS-matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). Those two soft-ionization methods integrate well with several different types of mass analyzers—time-of-flight (TOF), quadrupole ion-trap (Q-IT), quadrupole-TOF (Q-TOF), TOF-TOF, and Fourier-transform ion-cyclotron resonance (FTICR). Proteomics benefits significantly from the continuous improvements and developments of biological MS, which has become a conventional method for protein characterization. MALDI–TOF-MS and liquid chromatography (LC)–ESI–Q-IT–MS are the two most widely used instruments for protein identification; MALDI–Q-TOF–MS, MALDI–TOF–TOF-MS, and LC–ESI–Q-TOF–MS are also used. More than 500 protein spots on a 2D gel of a CSF sample have been analyzed with MS [6,9,12,16].

4.3.1. MALDI–TOF-MS

MALDI–TOF-MS is a simple, fast, and sensitive method to identify 2DGE-separated proteins, and can be operated in two different ways. MALDI–MS is used for peptide mass fingerprinting (PMF), and MALDI–post-source decay

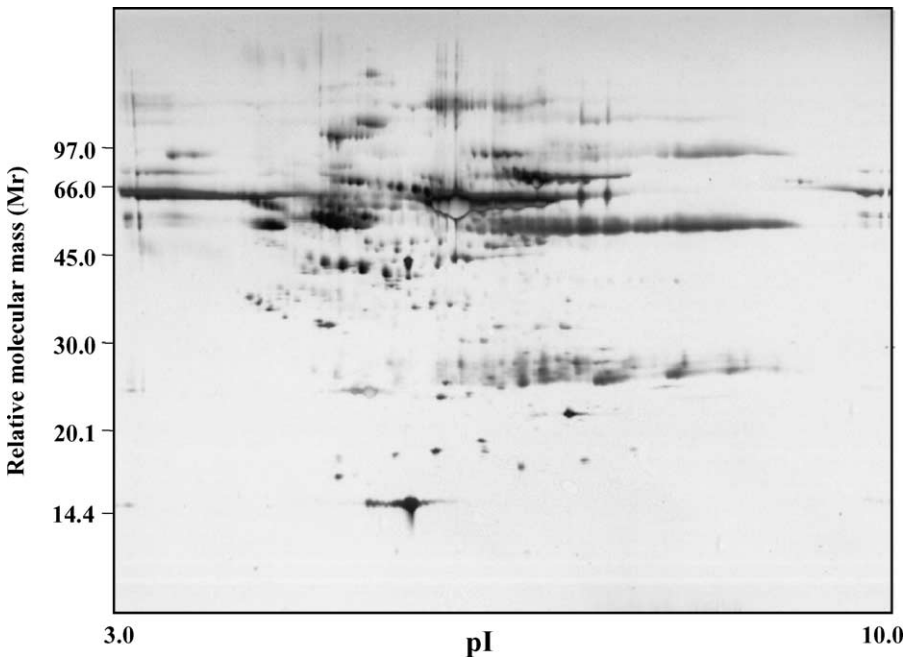


Fig. 1. A representative 2D gel (p*I* 3–10, linear) of a human CSF sample that was subjected to salt removal with a Bio-Spin column. Reprinted with permission from Yuan et al. [9].

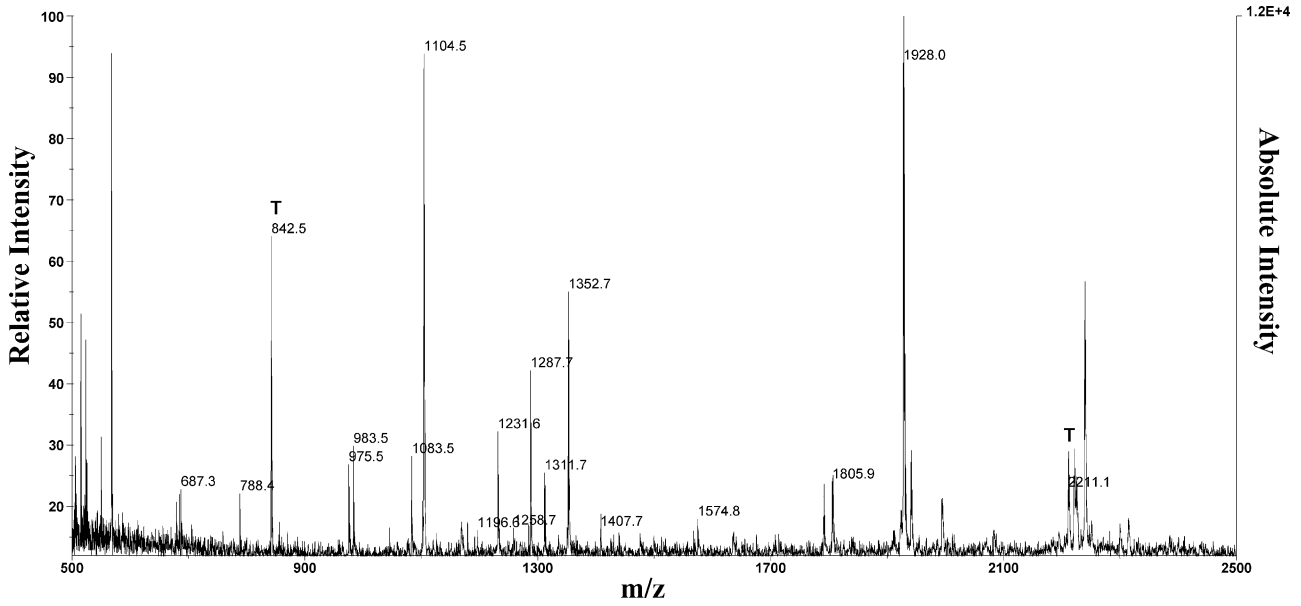


Fig. 2. MALDI mass spectrum of the mixture of tryptic peptides derived from 2DGE-separated protein–apolipoprotein A-IV. The masses from a trypsin autolysis product (T) were used for internal mass calibration. Reprinted with permission from Yuan et al. [9].

(PSD)–MS is used to obtain the amino acid-sequence data from a selected (within ± a few Da) precursor ion, usually the protonated molecule ion $[M + H]^+$, of a peptide. PMF and amino acid sequence data can be obtained on one instrument during the analysis, generally, of an unseparated mixture of tryptic peptides.

4.3.1.1. PMF. Fig. 2 from [9] contains a representative MALDI–TOF mass spectrum of the mixture of tryptic peptides that derived from a 2DGE-separated protein–apolipoprotein A-IV. MALDI–TOF-MS provides the $[M + H]^+$ ion of each component in a mixture. The trypsin autolysis products (labeled with T) were used for internal mass calibration to maximize the mass accuracy. The parameters that were used for the search of a protein database with PMF data are: enzyme: trypsin; species: *Homo sapiens*; p*I* range: ±1; M_r range: ±20%; missed cleavage sites allowed: 1; minimum peptide hits: 4; mass tolerance: ±100 ppm; considered modification: cysteine treated with iodoacetamide to carboxamidomethyl; and methionine in the oxidized form. Usually, several proteins could be found in any search. A gap of at least two matched peptides is required between the highest ranked and the next best candidate protein.

Teflon [23] was used as a MALDI sample support to improve the quality of a MALDI–TOF mass spectrum of a mixture of tryptic peptides. Compared with a stainless-steel surface and a conventional sample preparation method, a greater number of matched peptides were obtained. The reliability and success in the identification of low-level proteins was increased. Another hydrophobic surface, a wax-coated MALDI plate [24], was also reported for CSF protein identification.

4.3.1.2. PSD. Fig. 3 contains a MALDI–PSD mass spectrum of the $[M + H]^+$ precursor ion at *m*/*z* 1226.7, which was selected from the tryptic peptide mixture that derived from cystatin C [9]. Partial b-ion series and y-ion series, and several internal fragment ions, from that peptide were obtained. That amino acid sequence information from PSD confirmed the PMF result. However, for most peptides, a sufficient number, and intensity, of sequence ions cannot be produced by PSD for protein identification. Therefore, LC–ESI–MS is a superior alternative because it acquires the amino acid sequence of an LC-separated tryptic peptide.

4.3.2. LC–ESI–Q-IT–MS

LC–ESI–Q-IT–MS is the most-used method for protein characterization. Tryptic peptides are separated with high performance capillary LC; on-line ESI–Q-IT–MS (for example) produces amino acid sequence data. A more accurate and unambiguous peptide identification, and thus protein characterization, can be obtained from LC–MS/MS data, especially for those spots that contain more than one protein. It has been reported that ca. 40% of protein spots contain greater than one protein on the 2D gel of a simple organism such as yeast [26]. Fig. 4 from [25] illustrates the LC–MS identification of complement C4 and fibulin-1 in a 2D gel spot. Fig. 4(a) is the base-peak total ion chromatogram (TIC) of the tryptic peptide mixture. In that analysis, 11 peptides matched complement C4 and three peptides matched fibulin-1. Fig. 4(b) and (c) are the MS/MS spectra of a tryptic peptide that derived from complement C4 and from fibulin-1, respectively. Continuous b- and y-series ions were detected for those two peptides, and an unambiguous MS-characterization result was obtained for each protein.

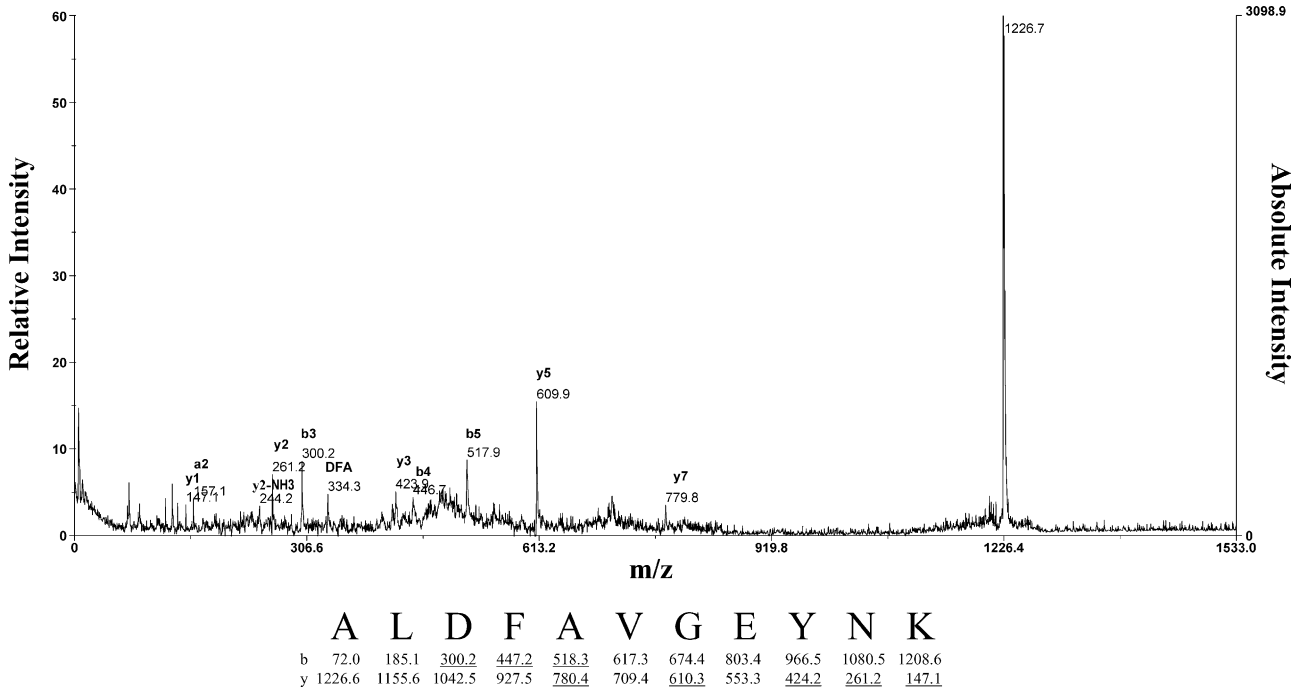


Fig. 3. MALDI–PSD mass spectrum of the $[M + H]^+$ precursor ion (m/z 1226.7) selected from the mixture of tryptic peptides that derived from 2DGE-separated protein–cystatin C. The amino acid sequence of the peptide is listed beneath the spectra. The masses of the b- and y-fragment ions are also indicated. The underlined numbers correspond to the fragment masses that were detected by MALDI–PSD–MS. Reprinted with permission from Yuan et al. [9].

4.3.3. LC–ESI–Q-TOF–MS

The hybrid instrument, Q-TOF, possesses high levels of mass accuracy and mass resolution. Isotopic peaks are well separated, and therefore, the charge state of an ion can be measured accurately. Peptide ions can be easily selected from co-eluting compounds for further MS/MS analysis because, generally, a peptide ion is multiply charged whereas ions from other compounds are generally singly charged. The high level of accuracy in the measurement of the mass of the precursor ion and product ions also significantly reduces the database search time. Some CSF proteins were identified with that method [12].

4.4. Protein databases

Protein databases are available for proteomics, and those complementary protein databases include NCBInr, Swiss-Prot, TrEMBL, Genpept, and PIR. Several search engines can be accessed freely via the Internet, and include ExPASy (Expert Protein Analysis System) Molecular Biology Server (http://us.expasy.org/), ProteinProspector (http://prospector.ucsf.edu/mshome4.0.htm/), and Mascot Search (http://www.matrixscience.com/search_form_select.html/). PMF data can be queried with PeptIdent (http://us.expasy.org/tools/peptident.html/), MS-Fit (http://prospector.ucsf.edu/ucsfhtml4.0/msfit.htm/), or Peptide Mass Fingerprint (http://www.matrixscience.com/cgi/searchform.pl?FORMVER=2&SEARCH=PMF/). Amino acid sequence data can be queried with MS-Tag (http://prospector.ucsf.edu/ucsfhtml4.0/mstaggd.htm/). Other commercial search engines include SEQUEST for the search of amino acid sequence data obtained from LC–ESI–Q-IT–MS, and ProteinLynx Global SERVER for the search of amino acid sequence data from LC–ESI–Q-TOF–MS.

5. Comparative CSF proteomics

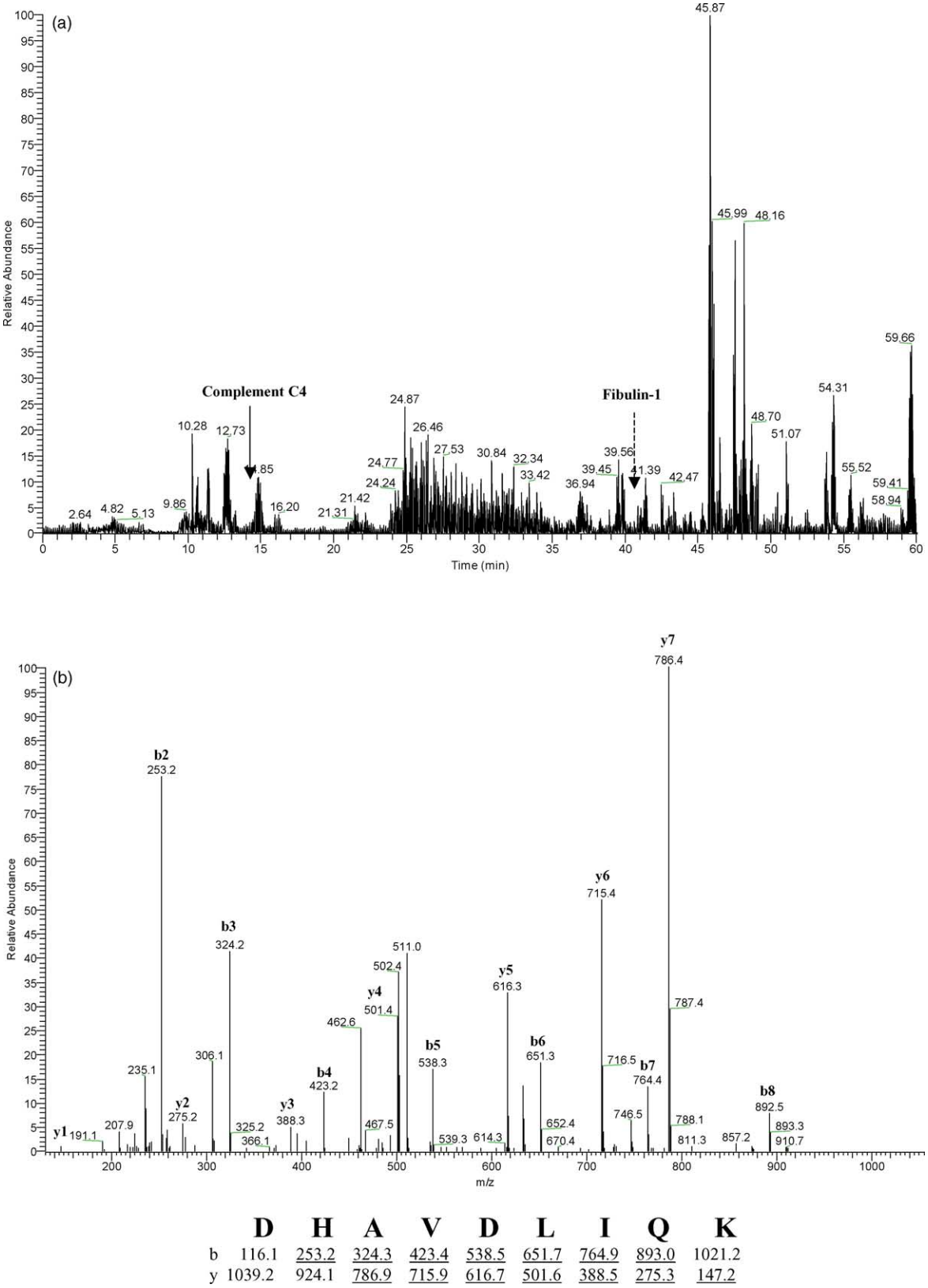
Comparative proteomics is used to locate differentially expressed proteins, which are used to elucidate the mechanism, at the molecular level, of a disease.

5.1. Between-gel reproducibility

A high-level of between-gel reproducibility is crucial for comparative proteomics. Terry et al. [11] investigated the reproducibility of 2D gels for the analysis of human lumbar CSF. CSF proteins were separated with an IPGphor system in the first dimension, and with a Protein-Plus™ Dodeca™ cell system in the second dimension. The proteins were visualized with silver-staining. Table 2 illustrates the reproducibility of the number of matched spots on the 2D gel of human lumbar CSF samples. The reproducibility of the volume of a spot

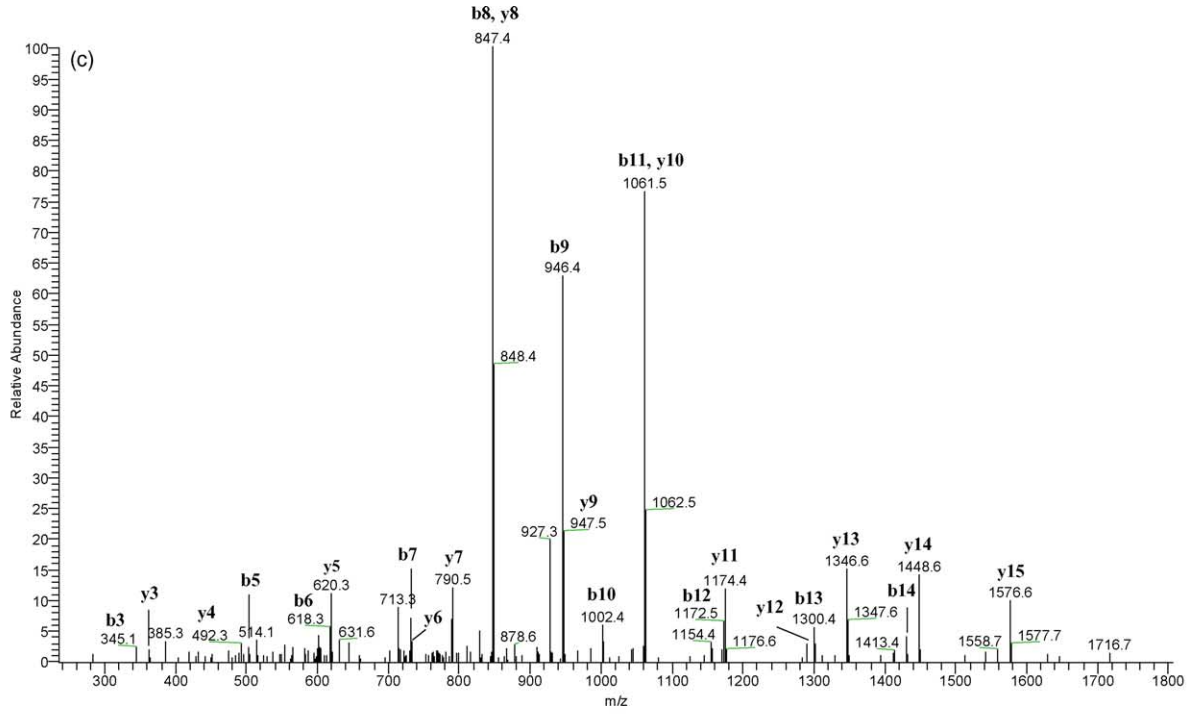
Table 2
Reproducibility of the number of the matched spots on a 2D gel of CSF [10]

	Within-sample	Within-group	Between-group
No. of matched spots	426–534	300–485	278–440
Matched percentage (%)	88–100	74–100	63–100



	D	H	A	V	D	L	I	Q	K
b	116.1	253.2	324.3	423.4	538.5	651.7	764.9	893.0	1021.2
y	1039.2	924.1	786.9	715.9	616.7	501.6	388.5	275.3	147.2

Fig. 4. LC–MS identification of two proteins in a 2D gel spot from a human lumbar CSF sample. (a) Base-peak total ion chromatogram of the tryptic peptide mixture. The arrows indicate the retention time of an MS/MS spectrum of a peptide that derived from complement C4, and an MS/MS spectrum of a peptide that derived from fibulin-1. (b) MS/MS spectrum of a peptide that derived from complement C4. (c) MS/MS spectrum of a peptide derived from fibulin-1. The amino acid sequence of each peptide is listed beneath each spectrum. The mass of each b- and y-fragment ions is also indicated. The underlined numbers correspond to the fragment masses that were detected by LC–MS/MS. Reprinted with permission from Yuan and Desiderio [25].



	S	Q	E	T	G	D	L	D	V	G	G	L	Q	E	T	D	K
b	88.1	216.2	345.3	446.4	503.5	618.6	731.7	846.8	946.0	1003.0	1060.1	1173.2	1301.4	1430.5	1531.6	1646.7	1774.8
y	1792.9	1705.8	1577.6	1448.5	1347.4	1290.4	1175.3	1062.1	947.0	847.9	790.8	733.8	620.6	492.5	363.4	262.3	147.2

Fig. 4. (*Continued*).

was also evaluated. The coefficients of variation for 61 spots ranged from 4 to 23%. It was suggested that several replicates from a sample should be analyzed simultaneously to reduce any variability in the 2DGE system.

Zhan et al. [27,28] statistically compared two second-dimensional electrophoresis instruments—the horizontal Multiphore II system and the vertical Dodeca™ Cell system. The Multiphor II system analyzes one gel at a time, and the Dodeca™ Cell system could analyze up to 12 gels at a time. The Dodeca™ Cell system demonstrated a better reproducibility of spot position and volume, and quantification for a wider linear range of protein concentration. Those results relate to the characteristics of a gel. The precast gel for the Multiphor II system is a gradient gel 12–14% (180 mm × 245 mm × 0.5 mm), and 40 mm in the stacking gel, therefore, the actual separation gel is 140 mm. The home-made gel for the Dodeca™ system is a homogeneous gel (190 mm × 205 mm × 1 mm). The reproducibility of a gradient gel generally is less than that of a homogeneous gel. First, a minor variability of the gradient in a gradient gel can alter the spot position on a 2D gel. Second, the spot on a homogeneous gel is round, and the spot on a gradient gel has a tail. The volume of a spot is based on the corresponding spot that derived from the original spot on a 2D gel. The image analysis software calculates, using the Gaussian model, more accurately the volume for a round spot. The home-made gel is larger and thicker than a precast gradient gel. The larger gel improves the gel resolution, and the thicker gel allows more tolerance in any background variation and a higher level of sample load.

5.2. Differentially expressed proteins (DEPs) and their functions

The 2D gels from samples of a control CSF with a CNS disorder [8,10,11,29–40] were compared with image-analysis software—PDQuest or Melanie, and several up- and down-regulated proteins were found. Table 3 correlates those CNS disorders, DEPs, and DEPs' functions. Some proteins have multiple functions, some are involved in multiple CNS diseases, and some regulate certain biological processes. Some proteins bind or transport other compounds, and therefore, mediate the biological activity of that compound, and also regulate all of the biological systems that use that compound. A CNS disorder might be a result of complex changes in those multiple biological processes and multiple biological systems.

6. Functional proteomics of CSF

An important aim of proteomics research is to understand, at the protein level, the basic molecular mechanisms that operate in the cell, CNS or CSF by an analysis of the dynamic

Table 3
Correlation of CNS disorders, differentially expressed CSF proteins, and proteins function

CNS disorders	DEP	Protein function	Ref.
1. CADASIL[a]	Complement factor B	Immuno-regulation	[7]
2. Creutzfeldt-Jakob disease	Apolipoprotein E	Lipid transport	[9]
	14-3-3 Protein isoforms	Activating kinase C; regulator of the cell signal processes	[34]
3. Pituitary tumor	Insulin receptor-related protein	Binding an insulin-related protein; Kinase	[11]
	Carbonic anhydrase I	Metabolism enzyme	[11]
4. Normal pressure hydrocephalus	Prostaglandin D2 synthase	Metabolism enzyme	[11]
	PRO2619	Unknown	[11]
5. Hydrocephalus	Vitamin D-binding protein	Binding and transport	[11]
	Serpine	Protease inhibitor	[11]
6. Alzheimer's disease	Apolipoprotein E	Lipid transport	[32,36]
	Apolipoprotein A-I	Cholesterol transport	[36]
	Apolipoprotein J	Clusterin	[36]
	Prostaglandin D2 synthase	Metabolism enzyme	[36]
	Retinal-binding protein	Retinal transport	[36,37]
	Kininogen	Interaction with β-amyloid	[36]
	α-1 Antitrypsin	Protease Inhibitor	[36]
	Cell cycle progression 8 protein	Regulator of cell proliferation	[36]
	α-1β-Glycoprotein	Unknown	[36]
	β-2 Microglobulin	Introduction of strong immune reaction	[37]
	Transthyretin	Binding thyroid hormone	[37]
	Ubiquitin	Unknown	[37]
7. Moyamoya disease	Cellular retinoic acid binding protein	Binding; regulator of biological activities of retinoic acid	[33]
8. Stroke	Heart-fatty acid binding protein	Fatty acid transport	[35]
9. Frontotemporal dementia	Granin-like neuroendocrine precursor	Endogenous inhibitor of prohormone convertase	[38]
	Pigment-epithelium derived factor	Regulator of glial function and proliferation	[38]
	Retinol-binding protein	Retinal transport	[38]
	Apolipoprotein E	Lipid transport	[38]
	Haptoglobin	Binding plasma hemoglobin	[38]
	Albumin	Binding and transport	[38]
10. Schizophrenia	Apoliprotein A-IV	Lipid metabolism	[39]
11. Primary brain tumors	*N-myc* oncoprotein	Regulator of transcription	[40]
	Low-molecular weight caldesmon	Binding actin and myosin	[40]

[a] CADASIL is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

proteome that results from the static genome. The comprehensive knowledge of each protein's function in a proteome will help to clarify those cellular, CNS or CSF processes. Functional proteomics is a methodology to study global proteins that have similar function [41]. Post-translation modification (PTM) processes play critical roles in a protein's function, and research on the PTM of a protein provides knowledge of its function.

6.1. Phosphoproteins

One of the most common and important regulation mechanisms is the reversible phosphorylation/dephosphorylation of proteins. Phosphorylation is implicated in many different cellular processes, such as metabolism, receptor activity, transcription, growth, and reproduction. Serine phosphorylation accounts for ca. 90% of all cellular phosphorylations, threonine phosphorylation for ca. 10%, and tyrosine phosphorylation for ca. 0.1%. For most proteins, the stoichiometry of phosphorylation is low. For example, the stoichiometry of tyrosine phosphorylation is <5% [42]. The methods that have been used to detect the global phosphorylation of proteins include immunoprecipitation, Western blotting, and MS-characterization. Yuan et al. [25] tested three different combination methods—1DGE plus Western blotting, immunoprecipitation plus 2DGE, and 2DGE plus Western blotting—to detect phosphotyrosine proteins in human lumbar CSF. Four proteins—kallikrein-6 precursor, complement C4 γ-chain, gelsolin, and ceruloplasmin precursor were identified—on a 2D Western blot as phosphotyrosine proteins.

6.2. Glycoproteins

Glycoproteins play important roles in cell–cell interactions, tissue morphogenesis, immune reactions, and pathologies such as cancer and inflammation [43]. CSF contains many glycoproteins, and each glycoprotein has a variety of

different glycosylated isoforms. For example, ca. 60 gel spots that corresponded to glycosylated isoforms of cellular prion protein were revealed by immunoblotting in studies of CSF and brain [44]. Hakansson et al. [45] reported the analysis of CSF glycoproteins that were visualized with a commercial glycoprotein detection kit. Selected glycoproteins were digested, each glycopeptide was analyzed with ESI-FTICR MS, and specific sites of N-glycosylation were assigned by infrared multi-photon dissociation.

7. Non-gel methods for CSF proteomics analysis

Compared with 2DGE, the critical advantages of non-gel methods are the time- and labor-saving aspects; the disadvantage is less resolution for a complex biological sample that contains thousands of proteins. In general, the tryptic peptides derived from all proteins in a sample are separated with LC or capillary electrophoresis (CE), and are analyzed with on-line MS.

7.1. Capillary LC–FTICR–MS

Reversed-phase capillary LC is a popular method to separate tryptic peptides, and is compatible with an ESI or nano-ESI source. FTICR possesses ultra-high levels of sensitivity, resolution, and mass accuracy. Ramstrom et al. [46] reported the combination of capillary LC with on-line FTICR–MS to analyze a CSF proteome. Thirty-nine proteins were identified in only 32 μl of CSF.

7.2. CE–FTICR–MS

Two CE techniques—capillary zone electrophoresis (CZE) [47] and capillary isoelectric focusing (CIEF) [48] were used to analyze the CSF proteome. Theoretically, CE has a higher level of separation efficiency and a shorter analysis time than capillary LC. However, the conventional solution (solvent; buffer) that is used for CE decreases the level of ESI ionization of peptides. The requirements for the CE for on-line MS could come at the price of decreased separation efficiencies. Thirty CSF proteins were identified with CZE–FTICR–MS [46], compared with 39 with capillary LC–FTICR–MS.

8. Conclusion

Even though significant research effort has been devoted to the proteomics analysis of CSF, much room for improvement exists to preferentially remove high-abundance proteins, to detect low-abundance proteins, and to characterize PTM CSF proteins. This research effort will provide a more comprehensive knowledge of the basic molecular mechanism of a variety of CNS disorders.

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