JOURNAL OF PROTEOMICS 74 (2011) 894–901

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Characterisation of the variation of mouse brain proteome by two-dimensional electrophoresis

István Földi, Géza Müller, Botond Penke, Tamás Janáky*

Department of Medical Chemistry, University of Szeged, Dóm tér 8., Szeged, 6720, Hungary

ARTICLE INFO

Article history:
Received 4 January 2011
Accepted 2 March 2011
Available online 13 March 2011

Keywords:
Brain proteome
Variation
Mouse strain
Two-dimensional electrophoresis

ABSTRACT

Neuroproteomics is aimed to study the molecular organisation of the nervous system at the protein level. Two-dimensional electrophoresis is the most frequently used technique in quantitative proteomics. The aim of this study was to assess the experimental and biological variations on this proteomic platform using mouse brain tissue. Mice are the most generally used lab animals for modelling human disease or investigating the effect of a drug-candidate or a treatment. Experimental design plays a crucial role in quantitative proteomics, hence understanding and minimizing the variables is essential. Our results indicate that the technical variance dominantly contributes to the total variance in mouse brain and the genetic background has a negligible effect on the total variation. The results also characterise the anticipated variation using mouse brain for proteomic study hence they should be useful for future experimental design in other proteomics laboratories.

1. Introduction

Proteomics is a multidisciplinary science that focuses on the analysis of the proteome, i.e. on aspects such as the expression, structure, interactions and posttranslational modifications of proteins [1]. Advances in technology have given us new tools to study the molecular organisation of the nervous system [2]. The prevalence of some neurodegenerative diseases has increased in the last few decades, so neuroproteomic studies have become more important and relevant.

Two-dimensional electrophoresis (2-DE) is the most frequently used technique in quantitative proteomics, which was first described in 1975 [3,4]. Many technical developments have been published which have helped to improve the reliability, reproducibility and resolution of 2-DE. The milestones of the improvements of modern 2-DE were summarised and reviewed by Görg et al. in 2009 [5]. Despite these developments, 2-DE remains a very manual, subjective and labour-intensive process [6]. Apart from gel-based quantitative proteomics, there are many gel-free, mass spectrometric techniques available which have been reviewed elsewhere [7].

In biological, medical, pharmacological and physiological research, proper experimental animal models are essential for the study of biological properties and mechanisms and to address scientific questions that have been raised [8]. Actually, seventy percent of all animals used in biomedical research are mice and rats. Over a thousand stocks and strains of mice have been developed, as have hundreds of mutant stocks that are used as models of human diseases. There are significant differences in the sensitivity between species because of their differing genetic backgrounds. This is the reason why different species are suitable or unsuitable for any given experiment [9]. Well-designed animal experiments should be in accordance with set experimental parameters, such as selection criteria, sample size and statistical analysis type [10]. In quantitative 2-DE the appropriate experimental design also plays a crucial role in the detection of significant and reliable protein expression differences. Components of a variation need to be taken into account in the experimental design phase. Technical variations arise from the sample preparation, electrophoresis, staining, image acquisition and image analysis; while the biological variation of genetic origin is influenced by growth/

* *Corresponding author*. Tel.: +36 62 342 890; fax: +36 62 545 971.
E-mail address: foldi@dnt.u-szeged.hu (I. Földi).

1874-3919/$ – see front matter
doi:10.1016/j.jprot.2011.03.006

housing conditions [11,12]. The total variation observed between individuals is the result of the biological variation and the technical variation. 2-DE is time-consuming and can be very costly, hence it is important to choose the appropriate number and type of replicates to reliably detect existing protein expression differences [13,14]. In addition, proteomics data derived from 2-DE analysis needs to be subjected to rigorous statistical analysis to avoid false conclusions [15]. The large amount of information derived from 2-DE analysis requires complex statistical procedures to extract and rationalise the useful information [16].

In this study, we analysed the 2-DE brain proteome profiles of NMRI and C3H/HEN mice. NMRI is an out-bred mouse stock that is used in many fields of research such as pharmacology and toxicology, and is useful in behavioural [17] and immunological [18] studies. C3H/HEN is an inbred mouse strain mainly used for immunological studies, as they display a lower cellular immune response compared to other mouse strains [19]. Our goal was to estimate the effect of the genetic background on the variation of mouse brain proteome. To achieve this, 2-DE brain protein profiles of littermate and non-littermate NMRI mice were studied and the variance components (technical and biological) were computed. The inter-experimental technical and biological variations were also evaluated. Moreover, the 2-DE brain proteome profiles of an out-bred mouse stock (NMRI) and an inbred mouse strain (C3H/HEN) were compared. Our results characterise the anticipated variation using NMRI or C3H/HEN and other mice brain tissue for 2-DE proteomic studies, hence they should be helpful for future experimental designs in other proteomics laboratories interested in neuroproteomics, especially using mouse models.

2. Materials and methods

2.1. Animals

Two-month-old male NMRI or C3H/HEN mice (30–40 g) were housed and used under the approval of the Animal Care Committee at the University of Szeged. Housing conditions were standardised in accordance with the generally accepted parameters, such as constant room temperature (22 ± 2 °C), humidity level (55 ± 5%), 12 h light/dark cycle, food and water available *ad libitum*.

2.2. Materials

Tris, urea, tiourea, 3-(cyclohexylamino)-1-propanesulphonic acid (CHAPS), dithioerithriol (DTE), 2-iodoacetamide (IAA), glycerol were purchased from Sigma-Aldrich (Budapest, Hungary), sodium dodecyl sulfate (SDS) and piperazine bisacrylamide were purchased from Bio-Rad (Hercules, CA, USA). Acrylamide was procured from GE Healthcare (Little Chalfont, UK).

2.3. Experimental design

2-DE brain proteome profiles of littermate or non-littermate NMRI mice were analysed. To estimate the technical variance two randomly selected samples were extracted and each of these extracts were analysed on parallel gels (n = 4). Thus, in this study the technical variance includes all of the variance components that are derived from the 2-D gel-electrophoresis, staining, scanning and software analysis. To determine the total variance, seven series of biological replicate gels (four series of littermate and three series of non-littermate) were run using brain tissue taken from each mouse (n = 4). Hence the total variance includes the components mentioned earlier, the sample preparation and the variation of independently prepared mouse brain samples, the so-called biological variance. Inter-experiments were performed using littermate mice (4 animals/group, *experiment 1*) or non-littermate mice (4 animals/group, *experiment 2*) to estimate the run-to-run variance, when the animals were born and about two months later sacrificed on the same day, and the sample preparation was performed in a short period of time. To analyse the potential protein expression differences between generations we compared the brain proteome profile of two consecutive generations of the same parentage (4 animals/group, *experiment 3*). In this case, there was a two-month difference between the two generations. A similar study was also made by comparing two non-littermate mice groups (4 animals/group, *experiment 4*). In this comparison, there was a two-month difference between the groups as well. Moreover, the brain proteome profiles of NMRI and C3H/HEN mice strains were compared (4 animals/group, *experiment 5*). Altogether, the present study includes eight groups of mice (four littermates, three non-littermates and one C3H/HEN; four gels/group) plus two series of technical replicates, of the whole forty gels that were made and analysed.

2.4. Sample preparation and 2D gel-electrophoresis

The mice were sacrificed by decapitation, then the brains were dissected and the forebrains were divided into hemispheres. The right hemispheres were homogenised in a 5× volume 2-DE lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTE) supplemented with a 1% (v/v) protease inhibitor cocktail (Sigma). The fresh homogenates were incubated on ice for 15 min then centrifuged at 14,000 *g* for 30 min at 4 °C. The supernatants were pipetted into clean Eppendorf tubes. Brain lysates were purified with a 2-D Cleanup Kit (Bio-Rad) according to the manufacturer's instructions. After purification, the final protein pellets were resolved in a 2-DE lysis buffer (100 μL/sample). The protein concentration was found via a Non-Interfering Protein Assay Kit (Calbiochem, Gibbstown, NJ, USA). A volume of samples containing 500 μg of total protein was supplemented with a 2-DE lysis buffer to a total volume of 450 μL plus 2.5 μL Bio-Lyte 3–10 buffer (Bio-Rad) and left on 24 cm, pH 3–10, NL IPG strips (Bio-Rad) for overnight rehydratation. Isoelectric focusing (IEF) was performed on an IEF cell (Bio-Rad) using a 24 h programme for a total of 67,000 Vh. After IEF, the strips were equilibrated for 2 × 10 min in an equilibration solution (6 M urea, 30% glycerol, 0.375 M tris pH 8.8, 5% SDS, trace bromphenol blue) supplemented with 20 mg/mL DTE. It was followed by a second equilibration for 2 × 10 min with the same solution without DTE, but with 50 mg/mL IAA. After equilibration, the strips were applied to the second dimension to separate proteins in a