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Journal of Chromatography B, 819 (2005) 115–129
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

The rat brain hippocampus proteome

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Received 6 September 2004; accepted 31 January 2005
Available online 19 February 2005

Abstract

The hippocampus is crucial in memory storage and retrieval and plays an important role in stress response. In humans, the CA1 area of hippocampus is one of the first brain areas to display pathology in Alzheimer's disease. A comprehensive analysis of the hippocampus proteome has not been accomplished yet. We applied proteomics technologies to construct a two-dimensional database for rat brain hippocampus proteins. Hippocampus samples from eight months old animals were analyzed by two-dimensional electrophoresis and the proteins were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The database comprises 148 different gene products, which are in the majority enzymes, structural proteins and heat shock proteins. It also includes 39 neuron specific gene products. The database may be useful in animal model studies of neurological disorders.

Keywords: Rat brain; Hippocampus; Proteome; Proteomics; Two-dimensional protein database; Mass spectrometry; Memory

1. Introduction

Proteomics finds a wide application in neuroscience nowadays [1–4]. It has mainly been used for protein screening in brain tissue in healthy and diseased states for the detection of drug targets and diagnostic markers [5–11]. Proteomics is the ideal tool for studying protein–protein interactions and post-translational modifications. Moreover, it has been applied in the generation of two-dimensional (2D) protein databases which are essential in the quantification of alterations in the protein levels resulting from the various disorders or the effect of external factors [2,12–16]. Previously, neuroproteomics has mainly been applied to the analysis of total brain tissue [17,18]. Such an analysis provides us with a general pattern of the proteins expressed in all brain regions. Detection of proteins involved in certain disorders demands the analysis of specific brain regions, the preparation of subfractions and the isolation of organelles, each containing a lesser number of total proteins [4,19].

The hippocampus is a cytoarhitecturally distinct structure folded into the cerebral cortex. It has been shown to be involved in the integration of information arriving from different sensory organs and associated areas and is essential for memory storage and retrieval, playing an important role in declarative memory. It has a high abundance of glucocorticoid receptors and through their actions it serves as an integral part of the feedback loop responsible for terminating glucocorticoid release during stress response [20]. In humans, the CA1 area of hippocampus is one of the first brain areas to display pathology in Alzheimer's disease [21]. Furthermore, magnetic resonance imaging (MRI) studies in humans have demonstrated hippocampal atrophy in certain diseases such as dementias, recurrent major depression and Cushing's disease [22]. There exist several proteomics studies for rat hippocampus [23,24]. Here we report a detailed two-dimensional database for the rat hippocampus proteome and the identification of the products of 148 different genes.

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doi:10.1016/j.jchromb.2005.01.037

2. Experimental

2.1. Materials

Immobilized pH-gradient (IPG) strips and IPG buffers were purchased from Amersham Biosciences (Uppsala, Sweden). Acrylamide/piperazine-di-acrylamide (PDA) solution (37.5:1, w/v) was purchased from Biosolve Ltd. (Valkenswaard, The Netherlands) and the other reagents for the polyacrylamide gel preparation from Bio-Rad Laboratories (Hercules, CA, USA). CHAPS was obtained from Roche Diagnostics (Mannheim, Germany), urea from AppliChem (Darmstadt, Germany), thiourea from Fluka (Buchs, Switzerland), 1,4-dithioerythritol (DTE) and EDTA from Merck (Darmstadt, Germany) and tributylphosphine (TBP) from Pierce Biotechnology (Rockford, IL, USA). The reagents were kept at 4 °C. Brain samples were derived from four Sprague–Dawley eight months old rats.

The animals were killed by decapitation and the brains were rapidly dissected and the hippocampus removed. The tissue was immediately frozen in liquid nitrogen and stored at −80 °C until use. The experiments were performed in accordance with the regional legal regulations.

2.2. Two-dimensional gel electrophoresis

Hippocampus tissue (0.1 mg) was suspended in 0.5 ml of 20 mM Tris, 7 M urea, 2 M thiourea, 4% CHAPS, 10 mM 1,4-dithioerythritol, 1 mM EDTA and a mixture of protease inhibitors (1 mM PMSF and 1 tablet Complete^{TM} (Roche Diagnostics) per 50 ml of suspension buffer) and phosphatase inhibitors (0.2 mM Na_2VO_3 and 1 mM NaF). The suspension was sonicated for approximately 30 s and centrifuged at 150 000 × g for 45 min. The protein content in the supernatant was determined using the Coomassie blue method [25]. The protein concentration was approximately 10 mg/ml.

Two-dimensional gel electrophoresis was performed as previously reported [26,27]. Briefly, samples were applied on immobilized pH 3–10 nonlinear gradient strips (18 cm). Focusing started at 200 V and the voltage was gradually increased to 5000 V at 3 V/min and kept constant for a further 6 h. The second-dimensional separation was performed in 12% SDS–polyacrylamide gels. The gels (180 mm × 200 mm × 1.5 mm) were run at 50 mA per gel, in an ETTAN apparatus (Amersham Biosciences). After protein fixation with 50% methanol, containing 5% phosphoric acid for 2 h, the gels were stained with colloidal Coomassie blue (Invitrogen, Paisley, Scotland) for 16 h. Excess of dye was washed from the gels with H_2O and the gels were scanned in an Agfa DUOSCAN densitometer (resolution, 400 dpi). The percentage of the spot(s) volume representing a certain protein was determined in comparison with the total proteins present in the 2D gel, using the ImageMaster software (Amersham Biosciences).

2.3. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS)

MALDI-TOF-MS analysis was essentially performed as described [4,28]. The spots were excised and destained with 30% acetonitrile in 50 mM ammonium bicarbonate and dried in a Speedvac evaporator. Each dried gel piece was rehydrated with 5 μl of 1 mM ammonium bicarbonate, containing 50 ng trypsin (Roche Diagnostics). After 16 h at room temperature, 20 μl of 50% acetonitrile, containing 0.3% trifluoroacetic acid were added to each gel piece and incubated for 15 min with constant shaking. The peptide mixture (1.5 μl) was simultaneously applied with the matrix solution (1 μl), consisting of 0.025% α-cyano-4-hydroxycinnamic acid (Sigma) and the standard peptides des-Arg-bradykinin (Sigma, St. Louis, MI, 904.4681 Da) and adrenocorticotropic hormone fragment 18–39 (Sigma, 2465.1989 Da) in 65% ethanol, 35% acetonitrile and 0.03% trifluoroacetic acid. The samples were analyzed in a time-of-flight mass spectrometer (Ultraflex, Bruker Daltonics, Bremen, Germany). Peptide matching and protein searches were performed automatically as described [29]. The peptide masses were compared with the theoretical peptide masses of all available proteins from all species. Monoisotopic masses were used and a mass tolerance of 0.0025% was allowed. Unmatched peptides or miscleavage sites were not considered for protein identification.

3. Results

3.1. Two-dimensional gel analysis

Hippocampus extracts from eight months old rats were separated by 2D electrophoresis and the protein spots were visualized following stain with colloidal Coomassie blue. Four samples were analyzed in duplicate. The protein profiles were similar with minor differences which were due to allelic differences and artifacts of the technology. Fig. 1 shows a representative example of the hippocampus proteins separated in a 2D gel, where 1 mg of total protein was applied. Approximately 2500 spots were counted in each 2D gel using the 2D ImageMaster software. The proteins were identified by MALDI-TOF-MS on the basis of peptide mass matching [30], following in-gel digestion with trypsin. The peptide masses were matched with the theoretical peptide masses of all proteins from all databases. Approximately 800 spots from two gels were analyzed. The analysis resulted in the identification of 148 different gene products (Table 1).

In Table 1, the SWISS-PROT accession numbers are listed as well as the abbreviated and full names of the proteins, the theoretical p*I* and MW values, as well as data from the mass spectrometry analysis, i.e. the numbers of matches and the protein amino acid sequence coverage by the matching peptides. The introduction of internal peptide standards to correct the measured masses allowed the use of very narrow windows of mass tolerance (0.0025%), increasing thus the confidence