Pak. J. Biochem. Mol. Biol. 2011; 44(2): 60-63

Differential expression of cytosolic and membrane associated proteins in different rat brain regions

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Abstract: The present research focuses on the expression profiling of brain regional membrane proteins and expression changes within two rat brain regions hippocampus and cortex. The protein extracts from rat brain regions (cortex n=5 and hippocampus n=5) were collected through subcellular fractionation, which allows the analysis of proteins in their physiologic and intracellular context. High-resolution maps of the hippocampal and cortex proteome were generated through sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and the protein spots were visualized with coomassie brilliant blue and silver staining methods. A 69, 58, 45, 35 and 25 kDa protein shows more than 2 fold up regulation in cortex membrane fraction as compared to hippocampus membrane fraction while increased expression was observed for 58, 55, 45, 35, 29 and 25 kDa proteins in cytosolic fraction of hippocampus in comparison to cortex. Further, protein components of 63, 55, 25 and 21 kDa have shown negligible expression in hippocampus membrane fraction. Moreover, a 50 kDa protein component is only expressed in cortex membrane and cytosolic fraction with decrease expression in the both hippocampal fractions. The restraining of these membrane proteins to a particular region provides clues about the involvement of proteins in catalyzing particular functions of each brain region. Moreover, the expression profiles of membrane and cytosolic proteins will help in elucidating the functional role not only in the particular region but also in a specified organelle. Further characterization through mass spectrometry will be helpful in identification and elucidation of the biological role of the expressed proteins.

Keywords*:* Membrane proteins, cytosolic proteins, hippocampus, cortex.
Received: April 8, 2011 **Accepted**: May 31, 2011
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INTRODUCTION

Proteomic analysis of brain is useful to comprehend the complexity of central nervous system and is also supportive to investigate its disorders. The advent of proteomics technology has great impact on brain associated disease diagnosis[1, 2]. Individual regions of brain are likely to have distinct composition[3] like 20% of the proteins are involved in protein binding processes, 10% in nucleotide binding, 10% in transport pathways and 8% in signal transduction[4].

The use of animal models has extensively been applied to study proteomic alterations and the possible role of different brain proteins. The mouse models provides insight into the molecular mechanism(s) through which various alterations and genetic mutations that initiates neurodegeneration and plays potential role in development of associated clinical and pathological phenotypes[5].

Membrane proteins holds great importance in mediating various cellular/molecular processes by functioning as ion channels, receptors, and ion transporters for receiving, conducting and transmitting signals across the membrane[6].

Although there is a tremendous advancement in the search for biomarker proteins for neurodegenerative and psychiatric disorders but there is still to come, ranging from enhanced protein isolation, purification, protein profiling, studies on post-translational and other modifications. Advances in proteomics hold great promise for improvements in the understanding, diagnosis and therapy of central nervous system disorders[7].

Functional characterization of expressed proteins and comprehensive understanding of these components in each region is essential to unravel various molecular and cellular mechanisms. The functional specification of brain regions could be the result of differences in protein expression which is evident by proteomic analysis that revealed different protein composition in distinct brain regions[6].

However, despite all the efforts made to identify functional involvement and biological functions of regional proteins, ambiguities are still present to unravel comprehensive proteome of each region. The present study focuses on the differential expression of several membrane and cytosolic proteins that will be helpful in evaluating the functional role in healthy and diseased circumstances and for a better understanding of the changes in molecular and cellular mechanisms in developing brain disorders.

MATERIALS AND METHODS

Materials

Acrylamide, bis-acrylamide, Tris base, glycine, potassium chloride (KCl), glycerol, CHAPS, dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), Pepstatin A, were purchased from Sigma (USA) and Complete® mini from ROCHE, Germany.

Animals

Male wistar rats, weighing 150–200g were used in the experiments. Prior to the experimental manipulation, the animals were cared for and handled in accordance with the standard guidelines for the care and use of laboratory Animals. The rat brains were rapidly removed after decapitation and placed on a chilled glass plate on ice and the cortex and hippocampus were separated and immediately frozen in liquid nitrogen and stored at -80^{o}C. All subsequent procedures involved in the tissue preparation were performed at 4°C.

Protein Extraction

The subcellular protein extracts of hippocampus and cerebral cortex were prepared as described previously[8]. Briefly, tissue homogenates were prepared in a lysis buffer (50 mM Tris, 100 mM KCl and 20% glycerol, protease inhibitors, pepstatin A, PMSF, CHAPS, DTT) and fractionated into cytosolic and membrane proteins using Optima™ L-XP Series ultracentrifuge (Beckman Coulter). The protein concentration of the two fractions was determined by BCA method (BCA assay, Pierce).

One dimensional electrophoresis (1-DE)

Protein profiling was carried out by SDS-PAGE ~~gels~~ according to Laemmli standard protocol[9]. Extracted membrane and cytosolic proteins were loaded onto 10% SDS-PAGE gels using Mini PROTEAN III (Bio-Rad) system. The gels were run on 25 mA/gel until the bromophenol blue dye front reached the end of the gel.

Staining and data analysis

After complete electrophoresis run the gels were taken out and stained with coomassie brilliant blue (Sigma) overnight and destained with 40% ethanol and 10% glacial acetic acid. Digital images of the gels were taken by gel documentation system (Bio-Rad). Protein bands quantification and intensity measurements were analyzed by Quantity One® 1-D analysis software (Bio-Rad).

RESULTS

Total protein content in cerebral cortex and hippocampus cellular fractions

The protein extracts of rat cerebral cortex and hippocampus were quantified by BCA method and expressed as µg of proteins/mg of brain samples. The protein yield of cerebral cortex and hippocampus was calculated as 4156±4.1µg of protein/mg and 1526±2.2µg of protein/mg respectively obtained from a total of five animals. The protein concentration in the membrane and cytosolic fractions of hippocampus and cortex shows a higher protein concentration of membrane proteins in cortex while there is a decrease in cytosolic protein concentration as compared to hippocampus (Figure 1).

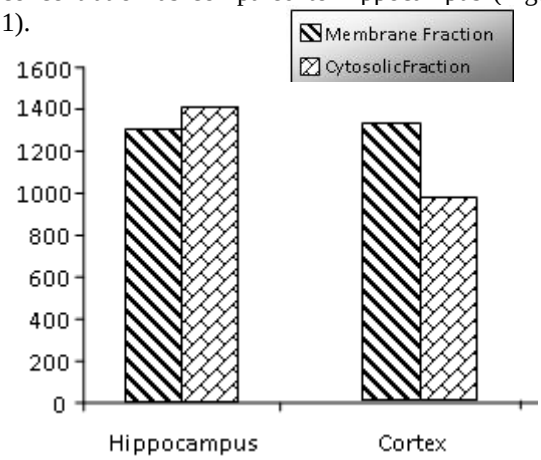

Figure 1: Total protein concentration of membrane and cytosolic fractions of rat hippocampus and cortex estimated by BCA assay method.

Protein exhibits regional differences in expression levels

The results obtained were based on three independent protein sample preparations resolved on SDS-PAGE (10%) and visualized by coomassie brilliant blue dye. Semi quantitative analysis by Quantity One® 1-D analysis software (Bio-Rad) reveals differentially expressed membrane and cytosolic proteins in studied rat brain regions (Tables 1 and 2). A total of 27 significant proteins were found to be expressed in the investigated regions. The protein profile represents the expression of low molecular weight proteins ranging between 69-20 kDa. A 69, 58, 45, 35 and 25 kDa protein shows more than 2 fold up regulation in cortex membrane fraction as compared to hippocampus membrane fraction while increased expression was observed for 58, 55, 45, 35, 29 and 25 kDa proteins in cytosolic fraction of hippocampus in comparison to cortex. Furthermore, protein components of 63, 55, 25 and 21 kDa has shown negligible expression in hippocampus membrane fraction. Moreover, a 50 kDa protein component is only expressed in cortex membrane and cytosolic fraction with negligible expression in the both hippocampal fractions (Figure 2). The total expressed proteins of the two regions are graphically presented in Figure 3.

DISCUSSION

Although different sub-regions of the human brain share anatomical connections, cell morphology and histological features, they differ considerably in

their vulnerability to different neurological and psychiatric diseases and function differently in health and disease conditions[10]. The parallel expression patterns of these proteins suggest their potential functional role in particular brain regions. Therefore, the protein profiles of specific brain areas, like cerebral cortex and hippocampus, is helpful in elucidation and identification of differentially expressed proteins within different brain regions.

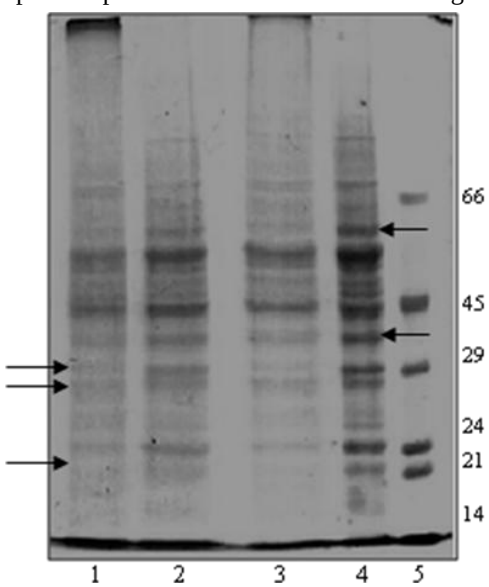

Figure 2: Protein fractions from cortex and hippocampus were separated on 10% gel and stained with coomassie brilliant blue. Lanes 1: hippocampus membrane fraction, 2: hippocampus cytosolic fraction, 3: cortex cytosolic fraction, 4: cortex membrane fraction and 5: protein standard marker expressed in kDa.

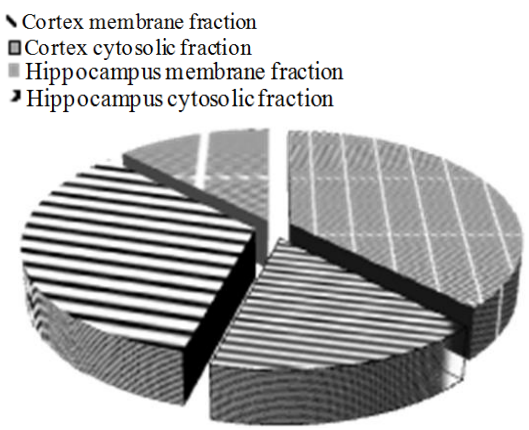

Figure 3: Graphical presentation of protein components expressed in membrane and cytosolic fractions of cortex and hippocampus. .

Differential expression of the hippocampal proteins with respect to cerebral cortex strengthens the idea that the functions related particularly with hippocampus like learning, memory formation[11] memory storage[12] retrieval of declarative memory[13] and integration of information arriving from different sensory organs and associated areas[14] could be altered. It is evident that most neurodegenerative disorders are region-specific and neuronal loss is observed in hippocampus and cerebral cortex in Alzheimer's disease[15, 16].

Table 1: Protein components differentially expressed in membrane fractions of cortex and hippocampus

Protein component	M_r (kDa)	Hippocampus membrane fraction	Cortex membrane fraction	Fold change
P1	69	++↓	++↑	2.0
P2	63	– –	++	
P3	58	++↓	++↑	2.0
P4	55	– –	++	
P5	50	– –	++	
P6	45	++↓	++↑	2.0
P7	41	++	++	
P8	35	++↓	++↑	2.0
P9	29	++	– –	
P10	25	– –↓	++↑	2.0
P11	21	– –	++	

Brain, with a multitude of different functions and having characteristic features of individual brain regions, protein expression analysis is very important to determine it's anatomical, physiological, and biochemical properties therefore investigations are often carried out on a single region or on subcellular structures[17]. Differential expression with 2 fold increase of five membrane protein components in cerebral cortex in comparison with hippocampal membrane proteins shows region specific expression change. While the presence of 50 kDa protein only in both cortex fractions also strengthens the idea of region specificity of particular proteins. The cytosolic fractions with different expression patterns also comprehend the same region specific functional involvement that distinguishes each region from others.

Table 2: Protein components differentially expressed in cytosolic fractions of hippocampus and cortex

Protein components	M_r (kDa)	Hippocampus cytosolic fraction	Cortex cytosolic fraction	Fold change
P16	58	++↑	++↓	2.0
P17	55	++↑	++↓	2.0
P18	50	– –	++	
P19	45	++↑	++↓	2.0
P22	35	++↑	++↓	2.0
P24	29	++↑	++↓	1.5
P25	25	++↑	++↓	2.0

The protein expression comparison between the two cellular fractions of different brain regions is helpful to understand the potential involvement and

associated functions of several expressed proteins in a particular brain region and how this difference in expression alters molecular and cellular processes. Furthermore, the overall data represents interesting and significant findings to construct regional maps of brain proteins at subcellular level. Further analysis using more advanced proteomic techniques like two-dimensional gel electrophoresis (2DE) and identification through Mass spectrometry would be helpful in complete characterization of the differentially expressed proteins in different brain regions that will enable the development of specific diagnostic markers and to unravel both genetic and environmental factors that precipitate and predispose to complex neurodegenerative and neurological disorders.

ACKNOWLEDGEMENTS

This work was supported by grant from Higher Education Commission grant no. 20-560/ R&D/07.

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