

Optimization of a Two-Dimensional Electrophoresis Protocol for Plasma Proteomic Profiling of Obese Schizophrenia Patients

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Abstract

The proteomic approach is particularly effective for studying the association between obesity and schizophrenia. It allows for a comprehensive analysis of the complete proteome, leading to substantial breakthroughs in biomarker discovery and drug development. Isoelectric focusing (IEF) and SDS-PAGE procedures are combined in the proteomic approach known as two-dimensional electrophoresis (2-DE), which separates proteins according to their isoelectric point and mass. This study aimed to investigate optimized conditions for the 2-DE technique by focusing on the selection of an immobilized pH gradient (IPG) strip. Protein extraction was performed on pooled plasma samples from 10 obese schizophrenia patients. The extracted protein samples were loaded onto two different pH (7 cm) IPG strips. The pH ranges between (i) 3 – 10 and (ii) 4 – 7. IEF was conducted following the PROTEAN IEF Cell System protocol, followed by SDS-PAGE. The resulting gels were stained with BioSafe Coomassie stain and washed with milliQ water. The stained gels were scanned, and the images were analyzed using PD Quest software. High-abundance proteins with a molecular weight range of 60 – 80 kDa were detected on both IPG strips. The results showed that using a pH 3 – 10 IPG strip, 245 protein spots were detected and distributed throughout the gel, with a notable concentration in the middle. Whereas using a pH 4 – 7 IPG strip resulted in the detection of 321 protein spots, indicating a higher quantity of protein spots with increased intensity. This is attributed to the improved fractionation of proteins resulting from the narrower and more focused pH range. Thus, it can be inferred that utilizing this pH range will yield optimal outcomes in protein separation and analysis. This study suggests selecting a pH 4 – 7 IPG strip is the recommended choice to achieve enhanced resolution and precise detection of protein spots in plasma samples from obese schizophrenia patients when employing the 2-DE method.

Keywords

Proteomics; 2-Dimensional Electrophoresis; IPG strip; Protein separation