High Resolution Proteome Analysis of Membrane Proteins by Fourier Transform-ICR Mass Spectrometry

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2-D gel electrophoresis using IPG gels [1] is a powerful, widely used separation method for complex protein mixtures extracted from cells, tissues or other biological samples. Although this technique is widely used in proteome analysis [2], particular problems can occur e.g. for post translationally modified proteins, strong basic or acidic proteins, or hydrophobic membrane proteins. In the present work we studied rat liver mitochondrial membrane proteins (porins) and neuronal cell-surface proteins from goldfish using a combination of classical protein purification techniques, 2D gel electrophoresis in gel digestion and FTICR [3] mass spectrometric peptide mapping. To improve the 2-D gel electrophoretic separation and recovery of membrane proteins, we studied a soluble recombinant human liver protein directly from crude E.coli lysate, which gave us the possibility to compare the results obtained for membrane and soluble proteins. The influence of different sample preparation and sample application techniques on the resulting two-dimensional gels, and the protein identification by mass spectrometric peptide mapping will be discussed. Following the same method we performed 2-D maps of prepurified proteins from rat liver mitochondria and the identification of membrane ion channel proteins (mitochondrial porins) via peptide mass finger printing. The FTICR-MS finger printing was also employed for several neuronal cell adhesion proteins (e.g. reggie-2) and samples from human brain of control patients and patients with Alzheimer's disease. The results obtained by database searching have been compared with data obtained using conventional MALDI-TOF analysis. By the application of high resolution FTICR-MS a dramatic increase in the identification selectivity and reliability was achieved.

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[3] A.G. Marshall et al. Mass Spectrom. Rev. 17, 1998, 1-35