

Proteomic analysis of rat liver microsomal proteins by a liquid-based two-dimensional chromatography combined with nano-LC-ESI-MS/MS

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A liquid-based two-dimensional separation system (2D-LC) method combined nano-LC-ESI-MS/MS was developed for analysis of hydrophobic rat liver microsomal proteins. The membrane proteins were separated on chromatofocusing as neutral pH range (pH 8.5-4.0) and basic range(10.5-7.4) for the first dimension followed by non porous reversed-phase HPLC for the second dimension. The detergent 2 % Trion X-100 was used for efficient solubilization of membrane proteins and removal of soluble protein. Nano-LC-ESI-MS/MS was used for large-scale identification of fractionated proteins. Among 140 proteins identified by nano-LC-MS/MS, about 48% proteins were hydrophobic proteins with more than one transmembrane domain (TMD) up to proteins with 15 TMDs. Approximately 28 % of identified proteins was located on membrane region. With respect to function, 28% proteins were identified to be involved in metabolism, 14% protein were related to cell signal and regulation and 14% proteins involved in transportation. The 2-D virtual image of pI vs. hydrophobicity could be used for differential display analysis. Thus, our 2D-LC system can provide large-scale analysis of hydrophobic rat liver microsomal proteins not only for the analytical separation but for the preparative separation with high resolution as compared to traditional gel methods. [supported by MOHW grant 03-PJ10-PG6-GP01-0002 (to YKP)]