PEPTIDE FRACTIONATION FOR PROTEOMIC STUDIES

Lázaro Betancourt, Aniel Sánchez, Jeovanis Gil, Jorge Fernández-de-Cossío, Yassel Ramos, Félix Alvarez, Vladimir Besada, Luis Javier González and Gabriel Padrón

Center for Genetic Engineering and Biotechnology. PO. Box 6162. Havana. Cuba.

Proteomics has evolved towards shotgun strategies based on multidimensional chromatography and mass spectrometry analysis (LC-MS/MS) of peptide mixtures derived from cell extracts. However, very complex peptide mixtures are obtained, limiting the detection of many of those peptides and impeding the identification of several proteins. Fractionation at protein or peptide level has been found to enhance protein identification.

Following this approach, we have developed three complementary methods (SCAPE) for selective isolation of peptides based on the derivatization of abundant functional groups (α and ε amino groups) to modulate the presence of positive charges (at acidic pH) and further separation by cation exchange chromatography or affinity chromatography.

These procedures have shown to be complementary, allowing high protein coverage. All of them have been developed for quantitative proteomics in combination with differential isotopic labeling. Particularly, one of these methods was implemented to analyze four proteomes simultaneously.

In addition we have developed a procedure for peptide fractionation by SDS-free polyacrylamide gel electrophoresis. Complex protein extracts separated by SDS-PAGE are trypsin digested and peptides further fractionated by SDS free-PAGE. Peptides migrate to the anode electrode in accordance with the charge-molecular mass ratio. Electrophoretic mobility of tryptic peptides increase for more acidic peptides and overlapping of peptides among fractions is lower than 15%. Detection of peptides improves substantially and hence, protein identification. An efficient method for peptide transfer to the second dimension was established while identified proteins increased 2.5 fold with respect to the direct analysis of non fractionated protein digest. The use of SDS for protein fractionation allows analysis of highly hydrophobic proteins and minimal protein loses. Analysis of a membrane protein extract from Neisseria meningitides allowed the identification of underrepresented proteins. The method could be also very useful for studying phosphorylated peptides.