Optimization of Trypsin Digestion and Mass Spectrometry Analysis for Shotgun Proteomics

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Two D gel electrophoresis (2 DGE) and shotgun proteomics (SP) are two main key technologies to compare protein expression. Combination of two methods is indispensable for large coverage of protein profiling, especially lipid-bilayer-bound proteins. SP is the technique that depends of protein solubilization and reproducible digestion with trypsin. For this purpose, many types of chaotropes and detergents have been used, but some of them are incompatible with trypsin digestion and mass spectrometry. We developed a protocol for trypsin digestion using a dilution of urea and detergent CHAPS that permits the use of the same sample for both techniques. The efficient extraction of proteins was tested with two cell lines T98G and U87MG, using 8 M urea and 1 % and 4 % CHAPS. Total protein content was determined by method of Bradford and protein expression levels by 2 DGE. The results showed that the recovery of total amount of protein in the cell extracts were equivalent (15 ± 3 µg/µl) (n=4) and number of spots in 2 DGE was 279±71 (n=4) for 300 µg protein loaded. Chicken lysozyme was used to test the interference of urea and CHAPS in trypsin digestion and mass spectrometry analysis, and it showed that the number of peptides produced during hydrolysis was not affected by the presence of urea and CHAPS and the ion intensities were decreased only 17%. We conclude that this protocol did not affect the quality of analysis and could be used for proteomic investigation. Supported by FAPESP and FINEP