Mining Low Abundance Proteins: Enhanced Proteome Profiling by Inhibiting Proteolysis with Small Heat Shock Proteins

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Proteolytic degradation is one of the most important problems in two-dimensional electrophoresis (2-DE). Loss of protein spots in 2-D gels due to residual protease activity is commonly observed when using immobilized pH gradient gels for isoelectric focusing. Three sHsps, IbpA and IbpB from E. coli and Hsp26 from S. cerevisiae, were found to be able to protect proteins in vitro from proteolytic degradation. Addition of sHsps during 2-DE of human serum or whole cell extracts of bacteria, plant A. thaliana, and human kidney cells allowed detection of up to 50% more protein spots than those obtained with currently available protease inhibitors. Here we identified the low abundance proteins that newly appeared in the gels of sHsps-treated proteome by using mass spectrometry. This finding may change the way proteome profiling is carried out by generally enabling the detection of many more protein spots. [This work was supported by a Korean Systems Biology Research Grant from the Korean Ministry of Science and Technology (2006-01691). Additional support was provided by the LG Chem Chair Professorship and the Center for Ultramicrochemical Process Systems.]