

Improving In-Solution Trypsin Digestion for Direct LC-MS Analysis

Protein characterization by mass spectrometry, such as protein identification and posttranslational modification, often required trypsin digestion to break down the proteins of interest into peptides. It is essential to detect unique peptides from the proteins of interest and not the autolysed peptides from trypsin. The trypsin peptides can complicate the analysis, as well as suppress the peptides from the proteins of interest. Even though The Facility uses sequencing grade trypsin, trypsin peptides still are inevitable. This is evident from the top trace of LC ion chromatogram (Figure 1) showing the most abundant trypsin autolysis fragment (m/z 421.9) eluted around 25.67 minutes.

Figure 1. Ion chromatograms

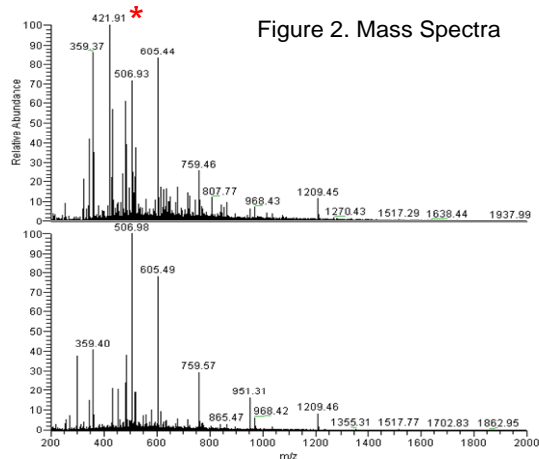
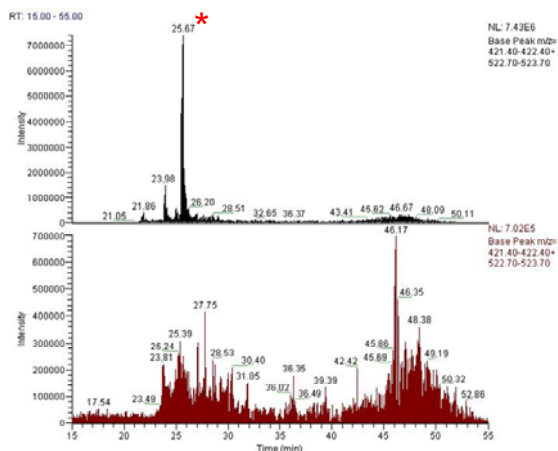


Figure 2. Mass Spectra

To eliminate the trypsin interference, the Facility has tested a new method to perform trypsin digestion of proteins in solution (not bound in gel). This product, NuTip from PolyLC (Bethesda, MD), is a unique pipet tip packed with immobilized trypsin beads. The sample solution, pH 7.5, is loaded into the NuTip and digested at 37°C. While proteins are being digested by trypsin immobilized on beads, trypsin does not contact other trypsin molecules, digesting and releasing the nuisance autolysed peptides. Using NuTip, the bottom trace in Figure 1 shows only the background contaminants. (Note the scales of the normalized chromatograms, the bottom trace only has 1/10 of absolute intensity as the top trace.) This reduction in autolysed peptides also clearly shows in the averaged mass spectra from the fraction around 25.67 minutes (Figure 2). The peptide at m/z 421.91 in the sample digested with the modified trypsin (top trace) is gone in that with immobilized trypsin (bottom trace).

Applications

This protocol will only work for in-solution digestion of proteins. Currently, applications that will be benefited most are “less complex” samples subjected to the direct LC-MS. For example, in pull-down experiments, eluted samples may not be very complex (20 or so proteins). Instead of running 1D or 2D gels followed by analysis of individual bands/spots, it is

possible to analyze the proteins in solution directly by LC-MS. Another application is for post-translational modification studies of pure proteins. In these cases, peptides from proteins of interest may be suppressed by trypsin fragments during mass spectral analysis. Occasionally, they have molecular weights very close to peptides from trypsin that could be overlooked. Using the immobilized trypsin considerably simplifies the LC-MS results that lead to better data analyses.

Summary

Using immobilized trypsin beads eliminate interfering peptides from trypsin when proteins are digested in solution. This potentially would enhance the quality of data analysis. LSUHSC Proteomics Core Facility now uses this technique as the default in-solution trypsin digestion for selected applications.