CID MS/MS

Peptide ion signals are first identified in MS1. These ions can be mass selected using a quadrupole or quadrupole ion trap. The selected ion can be fragmented by collision with inert gas (e.g., helium or argon). This is called collision-induced dissociation (CID). The type and number of fragment ions is determined by the amount of energy put into the molecule during the CID step. For low energy CID MS/MS, most fragment ions are dominated by the fragmentation occurring at the peptide bond. These ions are called either b- or y-type, but others can be observed as well. The b-type ion is a N-terminal portion of the parent peptide whereas the y-type ion is a C-terminal portion of the peptide. The resulting fragments will be analyzed by a 2nd mass analyzer and used for a database search to find a match. The more peptide fragments matched to the calculated peptide fragments in the library, the higher the scores obtained and the greater the confidence of identification. In our lab, both MALDI and ESI MS/MS can be performed on a hybrid quadrupole-TOF instrument (Applied Biosystems/MDS Sciex QStar), or the ion trap instrument Thermo Electron LTQ or Thermo Finnigan LCQ Deca).

Post-translational modifications can also be identified by CID MS/MS based on characteristic neutral losses (e.g., M - 98 in serine- or threonine-phosphorylated peptides) or product ions (e.g., m/z 216 corresponding to phosphotyrosine).

In-Gel Digestion

In-gel digestion is when protein separated by gel electrophoresis is subjected to digestion with a protease without elution from the gel. In-gel digestion was first introduced by the Suzuki lab[1] and further developed in our lab for successful applications[2,3]. The method was originally used for internal protein sequence analysis to regenerate oligonucleotides for PCR in order to clone a specific gene while the genome database was not available. For this purpose, a Lys-specific protease is more advantageous compared to trypsin because it generates longer peptide fragments that give a chance to perform PCR within a peptide, and it is a better enzyme because it is more specific and stable during digestion. With the advancement of the genome project and the development of mass spectrometry, trypsin became the dominant digestive enzyme because it generates more fragments for database matching with calculated peptide libraries. Unfortunately, V8 protease (specificity: Glu or Asp) does not work well with the in-gel digestion protocol. The protease, endoproteinase Asp-N does not work with the protocol. The advantage of in-gel digestion over the blotting methods is that one does not need to be concerned with the different blotting efficiencies of proteins to a membrane. Another advantage is that protein does not have to be completely pure. One must pay acute attention to ensure that the running buffer for SDS-PAGE is freshly prepared using the purest water and solid chemicals. The quality of the buffer significantly affects the recovery of the peptides. Another general caution is to wear gloves when you handle the gel in order to avoid keratin contamination. However, change gloves frequently so that the gloves themselves do not get contaminated.

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

proteomicfacility@mdanderson.org