EVALUATION OF PEPTIDE ALKYLATION SIDE-REACTIONS IN COMMON PROTEIN DIGESTION PROTOCOLS

N. Colomé [(1)], J.J. Bech-Serra [(1)], M. Monge [(1)], J.P. Albar [(2)], P. Consortium [(3)], F. Canals [(1)].

[(1)] Vall Hebron Institute of Oncology, [(2)] Centro Nacional de Biotecnologia-CSIC, [(3)] Spanish National Institute of Proteomics.

High-performance mass spectrometry instrumentation coupled with efficient chromatographic and electrophoretic separations has enabled the qualitative and quantitative analysis of global proteomes. Prior to MS analysis, proteins need to be broken up into peptides by enzymatic digestion. An effective digestion procedure includes a denaturation step (Urea, Guanidinium chloride, SDS, 2,2,2-Trifluoroethanol), a reduction of disulfide linkages (DTT, TCEP), an alkylation of sulfhydryl groups to prevent reformation of disulfide bonds (IAA, Iodoacetic acid), and finally, digestion with an endoproteinase (Trypsin, Lys-C).

In the last ProteoRed multicentric experiment, PME6, a qualitative analysis of a medium complexity sample, consisting of plasma major proteins, was performed by 20 different laboratories. In a centralized re-analysis of the results, using Mascot error tolerant search (second round), the phenomenon of overalkylation with IAA was clearly observed in the data from some laboratories. Overalkylation with IAA takes place during overnight digestions where excess of alkylating reagent remains in the medium. In this conditions S-alkylation of sulfhydryl groups as well as N- (Nterm, lysine, arginine, histidine) and O-alkylation (aspartic and glutamic acid) can occur.

We have evaluated the levels of overalkylation observed in the results of the different ProteoRed laboratories, and its relationship with the protocol and the IAA excess used to digest PME6 sample. Even though tryptic digestion is an essential step in proteomic analysis, protocols used vary substantially between laboratories. The results show that in some cases the conditions can be far from optimal. The observed overalkylation problems can affect to the results both qualitatively, to the number of identifications, and to any quantitative measurement, either based on intensity or in peptide count.