

Possible side-chemistries with guanidino labeling strategies of C-terminal lysine-peptides: Effect of different derivatization conditions

Olav Mjaavatten and Frode Selheim

PROBE proteomic platform, Dept of Biomedicine, University of Bergen, Norway

The most common approach in proteomic studies is the bottom-up strategy where purified proteins or complex protein mixtures are subjected to chemical or enzymatic cleavage. The vast mixture of peptides produced this way can then be separated chromatographically, and identified by mass spectrometric analysis. The typical enzyme of choice for mass spectrometry is trypsin because of its high specificity and biological activity, resulting in basic amino acids at the C-terminus of the digested peptides, e.g. lysine (Lys) and arginine (Arg). Tryptic digestion of proteins typically gives peptides in the range 500 – 2500 Da, which is ideal for mass spectrometry.

The ionization efficiency for Lys-peptides compared to Arg-peptides appear to be about the same for ESI (ElectroSpray Ionization), but not for MALDI (Matrix Assisted Laser Desorption Ionization) where ionization of Arg-peptides is favored. This is disadvantageous when looking for PTMs (Post Translational Modifications) where a high sequence coverage is needed. A common way to address this discrepancy is by guanidination of the ϵ -amino group of lysine, thereby making it more basic with increased affinity for protons. The two derivatization agents used in this process is O-methylisourea and Lys-Tag (2-methoxy-4,5-dihydro-1H-imidazole). Both of these guanidination procedures optimize the ionization of Lys-peptides for MALDI, but only the latter enhance peptide fragmentation and are therefore most useful during *de novo* sequencing. In addition, Lys-Tag can be purchased both as H4 and D4 labelled, and have been used to quantify differences in protein levels between two physiological states of a biological system.

In this work, we are investigating the selectivity of these two guanidination agents, identifying possible side-reactions at different derivatization conditions, and discussing the impact this may pose for quantification.