Abstract

Although the foundation of cellular processes is inherited in an organisms DNA, the true regulation of cellular behavior is often attributed to proteins. However, general protein expression does not entail protein activity, a property often regulated by post-translational modifications (PTMs). PTMs are important for controlling cellular processes such as translation, signaling and cell-cell interactions and it is crucial to understand their regulation to comprehend the complexity of a protein network. PTMs are usually added or removed by enzymes, many of which require certain metals for proper catalytic activity. Moreover, metals are involved in several cellular processes such as enzyme regulation and maintaining cellular integrity. Thus, inspiration from the use of metals in nature may present new ways to employ metals in biotechnology.

Protein phosphorylation is the most well-studied PTM because of its involvement in several diseases. However, mass spectrometry (MS) analysis of phosphopeptides is complicated by low phosphopeptide abundance, reduced ionization efficiency and facile phosphate loss upon collision induced fragmentation MS/MS. Thus, efficient procedures for phosphopeptide enrichment and methods for minimizing phosphate loss are in high demand. C-terminal peptide amidation is a very important PTM for hormonal- and neuropeptide activity. Hence, efficient ways of generating this modification is interesting to the pharmaceutical industry for development of new drugs.

The goal of the presented Ph.D.-project was to investigate the use of gallium and uranyl for PTM enrichment and protection or formation and the work was divided into two studies.

The first study focused on a metallo-organic complex with two gallium (Ga) ions that protects peptides from phosphate loss during MS. Here, we sought to develop a novel phosphopeptide enrichment strategy by targeting this Ga-complex via antibodies, β-cyclodextrin or molecularly imprinted polymers (MIPs). This study, presented in chapter 3, demonstrates that while the antibodies show strong affinity towards the Ga-complex, optimization is required for efficient enrichment. Initial results with β-cyclodextrin were promising but not reproducible, thus not confirming Ga-complex enrichment. Three MIPs were synthesized targeting the Ga-complex, and while one showed great promise for selective Ga-complex binding, we could not demonstrate binding of Ga-complex tagged species.

The second study focused on investigating the products arising from uranyl-catalyzed photocleavage of peptides. Upon sequestering of uranyl to a phosphopeptide, the ion induces cleavages in the peptide backbone when irradiated with UV-light. The aim was moreover to determine the nature of this reaction. The work is presented in chapter 4, which furthermore encompasses a manuscript describing the identification of the formation of C-terminal amidations upon uranyl catalyzed photocleavage.

Taken together, the work presented in this Ph.D.-thesis describes the use of the metals gallium and uranyl for either protection and enrichment or formation of PTMs. These results may be the foundation for novel phosphopeptide protection and enrichment in MS analysis and may suggest a novel reaction for introducing α-amidations into peptides.