Abstract

Methods of protein research based on mass spectrometry have become increasingly significant in the last few years. The proteolytic step is essential in a bottom-up approach, determining the success and relevance of identification and characterization of a protein and also the sequence coverage and spatial resolution of HDX-MS protocols. Conventionally used proteases however have their limitations and therefore efforts are being made to find alternatives that have, above all, different cleaving preferences and activity profiles. *An*PEP protease (*Aspergillus niger* prolyl endoprotease) has the advantages of both characteristic cleavage in the presence of proline (unlike many other proteases) and easy accessibility due to its commercial obtainability in large quantities.

This thesis examines the optimization of cleavage using *An*PEP protease obtained from commercially prepared Gluten Rid with Tolerase G. Firstly *An*PEP cleavage was tested on model protein (bovine carbonic anhydrase 2) under various conditions (temperature, pH, concentration of protease on a carrier or in solution). The objective was to find out how various conditions influence cleavage efficiency and preferences of this protease. It was discovered that *An*PEP protease is suitable for hydrogen/deuterium exchange coupled to mass spectrometry in both immobilized and solution form. On the other hand, due to non-specific and redundant cleavage at higher temperatures (20-50 °C), it is less suitable for methods of protein research like proteomics, chemical cross-linking or fast photochemical oxidation. Using recombinant protein (translocase of outer mitochondrial membrane 34), it was discovered that *An*PEP is applicable in the analysis of protein phosphorylation.