

## Abstract

PDI („potato cathepsin D inhibitor “) and NID („novel inhibitor of cathepsin D “) from potato (*Solanum tuberosum*) belong to the protein family of Kunitz inhibitors (I3 family, Merops database). These 20 kDa isoforms with the typical  $\beta$ -trefoil architecture inhibit aspartic and serine peptidases. In this thesis, the constructs for recombinant expression of PDI and NID in the yeast *Pichia pastoris* system were prepared and high-producing colonies were selected. Both proteins were identified in the cultivation media by mass spectrometry and N-terminal sequencing. A purification protocol for PDI with three chromatographic steps was designed. Analogous functional properties were demonstrated for the purified recombinant PDI and the native PDI isolated from a natural source. Analysis of the inhibitory specificity showed that PDI is a potent inhibitor of selected aspartic peptidases from the A1 family and serine peptidases from the S1 family, including a relevant enzyme of insect origin. This finding supports the hypothesis that Kunitz inhibitors are involved in plant defense against herbivorous insects. The inhibitors prepared within the project will be used for analysis of the reactive centers against target peptidases by protein crystallography.

*(In Czech)*

*Key words:* proteolytic enzymes, activity and inhibition of enzymes, aspartic and serine peptidases, peptidase inhibitors, Kunitz inhibitors, recombinant protein expression, chromatographic purification of proteins