Abstract

Proteases are involved in many physiological processes and their dysregulation is associated with various pathologies. Protease activity is effectively controlled by natural inhibitors. This PhD thesis is focused on the inhibitors of aspartic and serine proteases of animal and plant origin and provides the identification, biochemical characterization and structural description of their inhibition mechanisms.

Plant Kunitz inhibitors are produced as defensive proteins, and they are able to block activities of a broad spectrum of proteases. In this thesis, the digestive proteolytic system of the Colorado potato beetle, a herbivore pest of potato plants, was described with the help of functional proteomics. It was shown that aspartic and serine proteases from this herbivore are effectively blocked by two potato Kunitz inhibitors (namely PCDI, PSPI). Using structural analysis, novel types of reactive centers were identified on PCDI and PSPI molecules for the inhibition of aspartic protease cathepsin D and the serine proteases trypsin and chymotrypsin. The analysis of the reactive center on a PCDI with the crystal structure of digestive cathepsin D from the Colorado potato beetle explained the mechanism of their interaction.

Sphingolipids were identified as the first endogenous inhibitors of human cathepsin D. Sphingolipids are bioactive molecules with anti-cancer activity, and the mechanism of their action may include the regulation of cathepsin D, which is associated with cancer proliferation. Moreover, a proteomic probe based on a peptidomimetic inhibitor was developed for the detection of human cathepsin D, which is a prognostic marker in cancer.

The plant Kunitz inhibitor BbKI was identified as the most potent natural inhibitor of kallikrein-type serine proteases. BbKI is selective for the cancer marker kallikrein 4, and their interaction was described using a structural model. Furthermore, a proteomic tool for the detection of epidermal kallikreins was designed combining selective substrates and inhibitors, and evaluated on mice with genetically knock-out kallikreins.

To conclude, this PhD thesis provides important information about the specificity and inhibition mechanisms of aspartic and serine proteases, which can be used for the rational design of new inhibitors with biomedical and agricultural applications.