

## Functional and structural characterization of stefins and cystatins from pathogenic bacteria

Minca Ferlin

Department of Molecular and Biomedical Sciences, Jožef Stefan Institute, Ljubljana, Slovenia

Successful microbial pathogens have evolved a range of anti-immune strategies to overcome both innate and acquired immunity. This ability of pathogenic bacteria is due to multiple virulence factors acting individually or together in different stages of infection. Cystatins, inhibitors of papain-like proteases, have often been shown to be involved in host-parasite interactions as defense molecules or virulence factors. In the immune system, cystatins modulate cathepsin activities, antigen processing and presentation, expression of cytokines and nitric oxide. Therefore they can down-modulate the host immune response. Eukaryotic cystatins and stefins have been acquired by horizontal gene transfer (HGT) to a few ecologically closely located but taxonomically unrelated bacteria. Bacterial cystatins and stefins could play an important role in pathogenesis.

A detailed comparative and evolutionary genomic analysis of genes coding for stefins and cystatins in numerous prokaryotic genomes was performed. Analysis of promoter regions revealed transcription factors binding sites, which were previously found to influence expression of many virulence genes of bacterial pathogens.

Structural alignment has shown that bacterial stefins and cystatins have modified inhibitory domain. This indicates that inhibitory domain was adapted to inhibit a broader spectrum of cysteine cathepsins. In order to demonstrate the biochemical activity of bacterial cystatins we expressed the *Bacteroides fragilis* fusion inhibitor possessing chagasin and cystatin domains (BF1388) and the *Vibrio cholerae* stefin (VCA0935). We explored the inhibitory properties of recombinant proteins and determined their interaction constants with diverse cysteine proteases. VCA0935 and BF1388 were found to act as fast and tight binding inhibitors of papain and diverse cathepsins. Both have especially strong affinity to cathepsin L which is as an essential protease in antigen processing.

There are very few cases where protease inhibitors help pathogens in invading the eukaryotic hosts by inhibiting host proteases. Stefins and cystatins with inhibitory spectra for diverse eukaryotic cysteine proteases are especially suited to inhibit the numerous eukaryotic host cysteine proteases during infection. In this way, the bacterial stefins and cystatins could therefore function in the invasion and dissemination of the pathogens.