Abstract

Candida parapsilosis is an emerging human opportunistic pathogen causing a wide spectrum of potentially life-threatening infections in immunocompromised hosts. One of the most important virulence factors of *Candida* spp. is a production of secreted aspartic proteinases (Saps). Presented thesis is mainly focused on the study of secreted aspartic proteinase 1 (Sapp1p) of *C. parapsilosis*, its processing and secretion under variable conditions and by use of various experimental models.

Sapp1p is secreted by *C. parapsilosis* cells into the extracellular space as a completely processed and fully active enzyme. Experiments studying the *C. parapsilosis* cell wall (CW) confirmed the prolonged presence of completely processed Sapp1p on the cell surface (CW-Sapp1p). Proteolytic activity assay performed with the intact cells showed that CW-Sapp1p is proteolytically active prior to its release into the extracellular space and is capable of substrate cleavage. Biotinylation experiments with consecutive MS analysis revealed that CW-Sapp1p biotinylation is incomplete but saturable process, leaving partially unlabelled molecules. The accessibility of individual lysine residues in the Sapp1p molecule varied, with exception of four residues that were labelled in all of our experiments performed. The final step of Sapp1p secretion may not be a completely random process.

Secretion and processing of Sapp1p were further studied in *kex2Δ* mutant *S. cerevisiae* cells. *SAPP1* expression resulted in a secretion of Sapp1p flanking 39 amino acids on the N-terminus which was identified as pro-Sapp1p. Secreted pro-Sapp1p was not as readily autoactivated in acidic conditions as recombinant zymogen of Sapp1p produced in *E. coli*.

Finally, experiments with the expression of *SAPP1* driven by its own *pSAPP1* promoter in *S. cerevisiae* wt cells resulted in a secretion of fully processed Sapp1p together with its precursor in the presence of protein as a sole source of nitrogen in the cultivation media. Unlike in *C. parapsilosis* cells, Sapp1p was secreted also by *S. cerevisiae* cells in the presence of ammonium sulphate as a source of nitrogen. In this case, no precursor of Sapp1p was secreted alongside.

Despite the fact that *pSAPP1* seemed disconnected from regulatory network of *S. cerevisiae*, real-time PCR analysis confirmed differences in *SAPP1* expression levels which depended on the source of nitrogen used in the cultivation media, suggesting some sort of regulation after all.