Abstract

Aspartic proteases (APs) are crucial for diverse cellular processes. This thesis delves into the complexities of protein expression and characterization of vacuolar aspartic endoprotease Apr1p from *Candida albicans*, comparing it to its *Saccharomyces cerevisiae* ortholog, Pep4p.

Recombinant expression of Apr1p in *Escherichia coli* yielded the inactive proenzyme, proApr1p. Extensive refolding efforts failed to produce mature, active Apr1p, suggesting a reliance on intricate cellular machinery or specific post-translational modifications for activation. Attempts to leverage vacuolar enzymes or cell lysates for proApr1p activation were unsuccessful, potentially due to the fragility of isolated vacuoles and the complex mixture of enzymes in cell lysates.

Positive results emerged when Apr1p was expressed in *S. cerevisiae*, where fractionated cell lysates exhibited specific proteolytic activity at acidic pH after inhibiting serine and metalloproteases proteases. The eukaryotic system can probably produce active Apr1p. However, after preliminary small-scale experiments, upscaling of Apr1p expression in *S. cerevisiae* will be necessary in order to obtain sufficient amount of protein for further characterization.

A reciprocal gene swap experiment, exchanging *PEP4* in *S. cerevisiae* with *APR1* and vice versa, revealed surprisingly similar growth patterns and stress tolerance between swapped and wild-type strains. This suggests potential functional complementation between these orthologs under our chosen laboratory conditions. However, differences in nitrogen source utilization emerged, hinting at potential subtle distinctions in metabolic regulation.

Overall, this thesis contributes to our understanding of Apr1p protease expression, maturation, and function. It emphasizes the challenges in replicating complex biological processes *in vitro* and highlights the importance of exploring alternative protease activation and purification strategies. Additionally, the gene swap experiment underscores the potential for subtle functional divergence between orthologous proteases, warranting further investigation into their specific roles in cellular physiology and adaptation.

Keywords: *Candida albicans*, *Saccharomyces cerevisiae*, vacuole, peptidase, protease