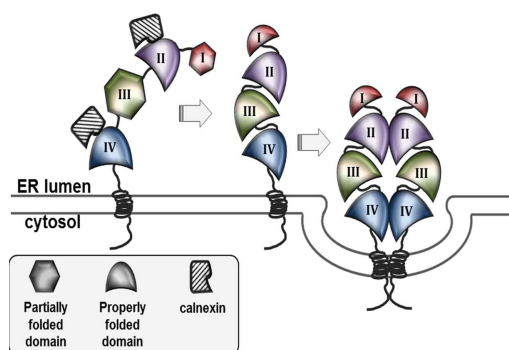


Expression and characterization of Lactase Phlorizin Hydrolase region III.

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Lactase phlorizin hydrolase belongs to the glycoside hydrolase family GH1. It is a brush border membrane protein responsible for lactose and phlorizin digestion. This protein has four regions; region I and region II act as intramolecular chaperones to aid in proper folding. Region III is enzymatically active, hydrolyzing phlorizin and other flavonoid and isoflavonoid compounds. The most remarkable capability of this region is its independence in its transport and enzymatic activity. Whereas, region IV contains enzymatic activity against phlorizin and lactose, it cannot be transported, and does not show any activity when isolated from the rest of the protein.



(Diekmann, Behrendt, Amiri, & Naim, 2017)

We expressed LPH region III (LPH III) in *Pichia pastoris*: removing the cleavage site, adding alpha-mating factor as secretion signal and a 6 Histidine chain as well as a TEV site for further purification. The protein was recovered from the culture by centrifugation, purified using nickel affinity, and then concentrated by filtration. Enzymatic analyses were performed showing a reduced hydrolytic activity against phlorizin in comparison with the protein isolated from intestine, to approximately 4%. Also, a screening test was done (Hauptmann-Woodward Research Institute, Buffalo) in order to establish initial conditions for crystallizing LPH III.