Abstract

Rutinosidases (α-L-rhamnosyl-β-D-glucosidases) from *Aspergillus niger* (*An*Rut) are glycosidases (EC 3.2.1) that catalyze the hydrolysis of the glycosidic bond between the aglycone and the disaccharide residue rutinose. The dual substrate specificity of this enzyme group describes the parallel activity towards the substrates rutin (carrying a rutinosyl disaccharide residue) and isoquercitrin (carrying a glucosyl residue). The active site of *An*Rut is more complex than that of other glycosidases and is composed of the catalytic amino acids Glu210 and Glu319 in the active-site cleft and a side tunnel. This untraditional structure with distinct interactions in the tunnel and active-site cleft is the probable reason for the enzyme exceptional substrate specificity. Through point or multiple mutations of the enzyme, we can modify its primary and secondary structure, thus causing a significant shift in substrate specificity.

The main goal of this thesis is the analysis of three distinct mutant variants of *An*Rut rutinosidase; their production, purification, and the study of the influence of the mutations on the substrate specificity of the enzymes. All variants were designed based on molecular modeling. The substrate specificity was determined by reactions of the mutant variants with previously unstudied substrates. The affinity of each variant towards the natural substrates rutin and isoquercetin was also determined using HPLC.

Keywords

diglycosidase; isoquercetin; mutagenesis; *Pichia pastoris*; rutin; substrate specificity; rutinosidase