Dual substrate specificity of *rutinosidase from Aspergillus niger* studied *on* Acylated Quercetin Glucopyranosides

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Rutinosidase is an enzyme that functions as a diglycosidase, facilitating the breakdown of rutinose (α-l-Rhap-(1-6)-β-d-Glcp) from rutin and other rutinosides. Additionally, rutinosidase can cleave β-glucopyranosides, such as isoquercitrin. Rutinosidase also exhibits robust transglycosylation activity and exceptional substrate specificity.

Rutinosidase from *Aspergillus niger* (AnRut) can cleave β-glucopyranosides acylated at the C-6 position of glucose (6'-O-acylisoquercitrin) with various acyl groups, including acetyl, benzoyl, phenylacetyl, phenylpropanoyl, cinnamoyl, vanillyl, galloyl, 4-hydroxybenzoyl, and 3-(4-hydroxy-3-methoxyphenyl)propanoyl [1]. 6'-O-acetyl, 6'-O-benzoyl, and 6'-O-cinnamyl derivatives of isoquercitrin, were also tested as transglycosylation substrates [1].

To confirm the accessibility of substrates to the active site of *An*Rut protein, a molecular docking study was performed [1] using a high resolution (1.27 A) experimental crystal structure of rutinosidase from *Aspergillus niger* K2 obtained from the Protein Data Bank (www.rscb.org; PDB ID: 6I1A [2]. Computational docking studies were performed using AutoDock Vina v.1.1.2 (La Jolla, CA, USA) [3] implemented in UCSF Chimera 1.17.3.[4]. The crystal structure of the protein was docked with twelve substrates.

Molecular modeling based on the crystal structure of AnRut revealed that large aromatic groups at the C-6' position of isoquercitrin obstruct the side tunnel of AnRut, which leads to its active site, thereby impeding the entry of the acceptor substrate for transglycosylation. This study further validates the previously proposed hypothesis [5] that the side tunnel in the AnRut structure acts as an access pathway for transglycosylation acceptors, and that blocking this tunnel can lead to the cessation of transglycosylation.

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