

Studies on the Substrate Specificities of α -L-Rhamnosidases of GH78 and GH106 towards α -L-Rhamnosyl and α -L-Mannosyl-linkages

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Title: Studies on the Substrate Specificities of α -L-Rhamnosidases of GH78 and GH106 towards α -L-Rhamnosyl and α -L-Mannosyl-linkages

(α-L-ラムノシルおよびα-L-マンノシル結合に対する GH78 と GH106 α-L-ラムノシダーゼの基質特異性に関する研究)

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論文内容の要旨

Thesis Summary

α -L-Rhamnosidases (α -L-Rha-ases, EC 3.2.1.40) are glycosyl hydrolases (GHs) that hydrolyze a terminal α -L-rhamnose residue from various glycoconjugates. These enzymes exist in a plethora of microorganisms, plants, and animals. α -L-Rha-ases possess broad substrate specificities towards α -L-rhamnose containing substrates hydrolyzing α -1,2, α -1,3, α -1,4, α -1,6 linkages. α -L-Rhamnose (6-deoxy-L-mannose) is a hexose, and extensively distributed in nature as constituent sugars in flavonoids glycosides, plant pigments and complex heteropolysaccharides. α -L-Rhamnose is also structurally similar to the rare sugar α -L-mannose. Rare sugars are defined as monosaccharides that are present in limited quantities in nature. Although the rare sugar α -L-mannose is structurally similar to α -L-rhamnose, there have been no reported studies of whether α -L-Rha-ases possess α -L-mannosidase activity. The proteins of the GH78 family of α -L-Rha-ases have been well studied owing to their potency for removing α -L-rhamnose from α -L-rhamnose-containing substrates as compared with the GH28 and GH106 families. This study used the GH78 α -L-Rha-ases; BsRhaA and BsRhaB from *Bacillus* sp. GL1, KoRha from *Klebsiella oxytoca* and SpRhaM from *Sphingomonas paucimobilis* from the GH106 family were examined whether these enzymes possessed α -L-mannosidase activity by hydrolyzing the substrate 4-nitrophenyl α -L-mannopyranoside. All α -L-Rha-ases exhibited both α -L-rhamnosyl and α -L-mannosyl-hydrolyzing activities with the GH106 SpRhaM α -L-Rha-ase from *S. paucimobilis* that showed relatively higher α -L-mannosidase activity as compared with the GH78 α -L-Rha-ases. The AnRhaE from *Aspergillus nidulans* (GH78) was depicted to be the first eukaryotic α -L-Rha-ase that is phylogenetically distant to any reported filamentous fungal origin but more closely related to bacterial α -L-Rha-ases of class A BRhaA from *Bacillus* sp. GL1 with similar α -L-mannosidase activity.

Next, the α -L-mannosidase activity of SpRhaM (GH106) and the influence of pH on its activity toward two substrates, pNP- α -L-rhamnose and pNP- α -L-mannose, was examined. The enzyme exhibited an optimum pH of 7.8-8.0 toward pNP- α -L-rhamnose with highest activity observed at pH 8.0. By contrast, the highest activity toward pNP- α -L-mannose was observed at pH 7.0 and the α -L-mannosidase activity of SpRhaM decreased by 30% at pH 8.0. Thus, the α -L-mannosidase

activity of SpRhaM depends on pH, and is highest (6.5%) at pH 7.0 (Tris-HCl buffer). A substrate-dependent shift of optimum pH was also observed for several glycosidases such as mammalian α -amylases and *Bacillus* endo-1,4- β -glucanase. In the presence of larger substrate α -L-mannose, C-6 position of α -L-mannose induces the conformation change of amino acids residues and this change might affect the pKa of general base catalyst (E594 of SpRhaM).

The site-directed mutagenesis experiment was conducted to residues near the C-6 position of L-mannose. Five mutants (L281E, L281K, W335A, W335R and F338A) were predicted that are in the vicinity of the C-6 position of α -L-mannose with the assumption of exhibiting high α -L-mannosyl-hydrolyzing activity. These mutants showed significant decreases in α -L-rhamnosyl and α -L-mannosyl hydrolyzing activities as well.

Recent studies have reported that heteropolysaccharides isolated from the fruiting bodies of mushrooms contain terminal L-mannose residues. These findings suggested that some natural substrates such as these fungal polysaccharides have a backbone α -1,4- linked L-mannopyranose residues and that α -L-mannose-containing polysaccharides may be widely distributed in nature. The results presented revealed great potential for the SpRhaM α -L-Rha-ase of the GH106 family that may acts on α -L-mannose containing natural substrates. Therefore, α -L-mannosidase will be useful for the identification and determination of structure of α -L-mannose-containing polysaccharides from natural sources.