

The Loop Regions and Substrate Specificity of GH 27 Family α -Galactosidases



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Abstract

α -Galactosidases as natural biocatalysts have important application value in various industries. In this paper, we reviewed their physiological functions, biological sources, classification, and protein structure and substrate specificity relationships of Glycoside hydrolase (GH) family 27, aiming at providing references for related experiments and studies.

Keywords: GH27 α -Galactosidase; Loop region; Substrate specificity; Thermo stability; Stability at low pH 29

Abbreviations: GH: Glycoside Hydrolase

Introduction

Galacto-oligosaccharides (i.e. stachyose and raffinose) commonly exist in food and feed and are indigestible by human and animals, thereby causing flatulence, gastrointestinal disturbance and low feed efficiency. [1] α -Galactosidases (EC. 3.2.1.22) has hydrolysis ability to degrade these anti-nutritional factors, decrease the viscosity of the diet, reduce the occurrence of diarrhoea, destroy the structure of the cell wall of the plant, promote the nutrition release, improve the utilization efficiency of the nutrients in the feed, increases lean meat rate, enhances immune function and disease resistance of animals. [2,3] α -Galactosidases has great application value in industrial processes of feed, food, and beet sugar production. α -Galactosidases are widely distributed in fungi, bacteria, plants and human (www.cazy.org). Fungal α -galactosidases have maximal activity at pH 3-5, but bacterial α -galactosidases are the optimal pH of 6-7.5.1, [4-6] Some thermostable α -galactosidases have been identified from thermophilic fungi, such as *thermomyces lanuginosus*, *Talaromecys emersonii*, and *Rhizomucor miehei*. [7-9] Due to high processing temperatures and acidic environment of the gastrointestinal tract, the highly efficient, thermostable and acidophilic α -galactosidases with broad substrate specificity is of great interest. [10] Base on the sequence similarities, α -galactosidases are divided into Glycoside Hydrolase (GH) families 4, 27, 36, 57, 97, and 110. [11] Most fungal α -galactosidases belong

to GH27 and have conserved YLKYDNC catalytic motif and DD(G/C)W binding motif. [12,13] The resolved crystal structures of two GH27 α -galactosidases from *Trichoderma reesei* (1t0oA) and *Saccharomyces cerevisiae* (ScAGal, 3LRK) share a (β/α)₈ barrel fold and a retaining reaction mechanism. [13,14] Sequence analysis indicated Loops 1, 2, and 4 of α -galactosidase from *T. reesei* and corresponding loops 1-3 and 6 from ScAGal create new binding sites for formation and breakdown of a covalent glycosyl enzyme intermediate. [13,14] In our study, two α -galactosidases Gal27A and Gal27B of family GH27 from the thermophilic *Neosartorya fischeri* had similar tertiary structures but varied in loop regions and substrate specificity. [15,16] Gal27A had far separate loops and showed higher activity towards raffinose, which was 4.9- and 3.8-fold for that of melibiose and stachyose.

[15] Whereas stachyose was a preferred substrate for Gal27B with closely proximate loops and its activity to stachyose was 9.6- and 4.4-fold of melibiose and raffinose, respectively. [16] Introduction of loop 4 of Gal27A into Gal27B elevated the activity to raffinose and broadened the substrate specificity (data not shown). Kinetic analysis of prolyl oligopeptidase indicated the loop splitting decreased the affinity of the enzyme to the substrate. [17] Site-directed mutagenesis revealed loops facing the active site of prolyl oligopeptidase can regulate the substrate gating and specificity.

[18] Thus the flexibility and motility of loops are presumed to be involved in enzyme-substrate interactions. The transformation of the zymoproteins is an important source to obtain excellent zymoproteins for various industries. With the development and accumulation of structural biological information of protein structure and function, protein rational design will inevitably become an important means to improve the properties of enzyme proteins.

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