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Molecular Analysis of α-Glucosidase from Microorganism

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Abstract

α-Glucosidase contributing α-glucan metabolism in microorganisms is characterized by the variety in substrate recognition. Recent studies on microbial enzymes show that α-glucosidases are divided into two groups, family I and family II, in which family I enzymes have four conserved catalytic-regions of α-amylase family. The presentation focusing on the difference of the α-glucosidase families reviews i) the catalytic amino-acid residues of nucleophile and acid/base catalyst, and ii) the molecular evolution of two families.

Introduction

α-Glucosidase (EC 3.2.1.20) is an exo-type hydrolase to release α-glucose from the non-reducing end of substrate (1). In microorganisms, α-glucosidase contributes the metabolisms of α-glucans and their oligosaccharides, supplying the glucose utilized for the energy production. α-Glucosidase is clearly distinguished from glucoamylase, an exo-type hydrolase, that liberates the β-glucose. The essential ionizable groups in the active site were kinetically determined to be two carboxyl groups, $-COO^-$ and -COOH (2). Various types of α-glucosidases are observed in microorganisms, as well as in animals and plants, and they are characterized by the diversity in the substrate recognition (3). The substrates are the oligosaccharides (α-glucobioses and malto-dextrins), the hetero-saccharides (sucrose and aryl α-glucosides), and the α-glucans (soluble starch and glycogen). >From their substrate specificities, the α-glucosidases could be classified into two groups at least. The first group, distributing in the insect (4), brewer's yeast (5) and bacteria (6), attacks heterogeneous substrates, such as sucrose and aryl α-glucosides, more quickly than maltooligosaccharides, and shows no or less activity to α-glucan (so-called real "α-glucosidase" to recognize α-glucosyl structure). The second group, distributing in animal (7), plant (8) and mold (9, 10), has higher activity toward maltooligosaccharides than heterogeneous substrates, and hydrolyzes the α-glucans (so-called "maltase" to recognize maltosyl structure). The difference in substrate recognition implies that their catalytic site structures are also distinct. It is of interest to learn the relationship between the catalytic action and the structure of the active site in two α-glucosidase groups.

Catalytic Amino-Acid Residues

1. Identification of Nucleophile

A mechanism-based irreversible inactivator, suicide substrate, is an effective chemical to identify the catalytic amino-acid residue. Conduritol B epoxide (abbreviated as CBE) is known as a suicide substrate for α-glucosidase (11-16) and labelled the catalytic residue of nucleophile ($-COO^-$). It was

reported that CBE bound specifically to β-carboxyl group of essential Asp in the active site of mammalian α-glucosidase (Table 1), such as Asp-505 and Asp-1249 of rabbit intestinal sucrase-isomaltase complex (13) and Asp-518 of human lysosomal α-glucosidase (14). CBE was found to modify the Asp-469 of sugar beet α-glucosidase (15). Mammalian and plant enzymes belong to the second group. There has been no report on the group II member from microorganism.

Table 1. Catalytic Reagions Conserved in α-Glucosidase Families I and II

α-Glucosidase Family I				
Enzyme	Region I	Region II	Region III	Region IV
S. cerevisiae	106-DLVINH	210-GFRIDTAGL	276-EVAH	344-YIENHD
B. sp. (SAM 1606)	113-DLVANH	210-GFRMDVINA	271-ETGG	340-YWTNHD
HBGase	116-DLVPNH	225-GFRIDAVPH	298-EAFS	355-VMGNHD
A. oryzae α-anylase	117-DVVANH	202-GLRIDTVKH	230-EVLD	292-FVENHD

α-Glucosidase Family II		
Enzyme	Region A	Region B
SPGase	476-SGIWTDMSEPSSF	641-GAHWLGDNHSLW
ANGase (P2)	219-DGVWYDMSEVSSF	388-AGHWGGDNYSKW
Human lysosomal acid	513-DGMWIDMNEPSNF	610-AGHWTGDVWSSW
Rabbit intestinal (isomaltase)	500-DGLWIDMNEVSSF	598-AAHWLGDNTATW
Rabbit intestinal (sucrase)	1389-DGLWIDMNEPSSF	1494-AGHWLGDNYARW
Sugar Beet	464-DGIWIDMNEASNF	562-TAHWTGDNAATW

Underlined D, the catalytic Asp identified experimentally.

We selected α-glucosidases from *Aspergillus niger* (abbreviated as ANGase) as a target enzyme, since its property was very interesting. ANGase consisted of two subunits, namely Pl of small size and P2 of large size (17). It was interesting to learn the location of the catalytic site in two components, since the separation of enzymatically active subunits was tried, however, such attempts were unsuccessful. Detailed kinetic analysis indicated that CBE modified the catalytic group (-COO^-) of ANGase (16). CBE was found to bind Asp-224 of P2 subunit of ANGase, which acts as the nucleophile in catalytic reaction. The sequences containing catalytic Asp in second α-glucosidase group shows high similarity (the average homology, about 60%).

The same approach was also done to honeybee α-glucosidase (abbreviated as HBGase) of the first group, and CBE modified one Asp, a nucleophile of HBGase (16). The portion containing the catalytic residue of HBGase is also shown in Table I with those of first group enzymes. The sequence corresponds to the region II of α-amylase family. Even if CBE bound to Asp in specific regions of first and second α-glucosidase groups, there is low homology of about 20 % between sequences of two groups. The difference in substrate recognition and catalytic structure suggests that α-glucosidases may be divided into two families (1): "family I" composed of the first group α-glucosidases and "family II" composed of the second group α-glucosidases. Moreover, the whole primary structures of the two groups also support the classification, since the high homology is observed in the enzymes belonging the same family, but no or less between the other family members (1).

2. Identification of Acid/Base Catalyst

The catalytic carboxyl group of acid/base catalyst (-COOH) is a proton donor to oxygen atom in the glucosidic linkage of substrate and also a proton acceptor from water molecule in the hydrolytic reaction. Family I enzymes have the highly conservative regions observed in α-amylase family (18), meaning that the catalytic carboxylate residue is Glu in region III and/or Asp in region IV which correspond Glu-230 and Asp-294 in *Aspergillus oryzae* α-amylase (19), respectively.

The acid/base catalyst in family II enzymes has not been identified. We have cloned an α-glucosidase gene from *Schizosaccharomyces pombe*, and expressed it in *Saccharomyces cerevisiae* cells (20). The α-glucosidase, termed as SPGase, was found to be a typical family II enzyme. Until now more than 20 α-glucosidases belonging family II have been cloned. After comparison of the reduced amino acid sequences, the completely conserved Asp and Glu in SPGase were replaced by different amino acids, such as Asn, Gln, and Ala (20). Nine acidic amino acid residues, seven Asp and two Glu, were perfectly conserved. The enzyme activity in transformants was analyzed, and the expressed mutant enzymes, D481N/E/A, E484Q/A and D647N/E/A, were found to have no activity. The Asp-481 was thought to be an essential residue acting as a nucleophile which was modified by CBE specifically. The properties of Glu-484 mutants were interesting. E484Q/A lost the hydrolytic activity, however, E484D remained about 10% activity of wild type, implying that the carboxyl group is needed to be in this position for hydrolytic reaction. E484A, which did not catalyze hydrolysis, showed the hydration activity of D-glucal. The function of the Glu-484 has remained obscure. The hydrolytic activity of the mutant SPGase substituted Asp-647 with Asn, Glu or Ala were missing, which indicates that the Asp-647 is a catalytic amino acid residue as a proton donor (20). The sequences including the essential residues of family II enzymes are shown in Table 1. In this table, the region A contains Asp-481 and Glu-484, and the region B contains Asp-647.

ANGase is a glycoprotein (17, 21) which has 16 *N*-glycosylated motives (Asn-X-Ser/Thr, sequon) (22), and Edman degradation analysis revealed that Asn residues in 15 sequons were modified and only Asn-395 in P2 subunit was free from binding of sugar chain (23). The sequence, Asn-Tyr-Ser (395 to 397), is in region B, and the neighboring Asp-394 corresponds to Asp-647 of SPGase. The reason why the Asn-395 is not *N*-glycosylated may be due to the exclusion of impediment for the catalytic reaction with sugar chain in the active center, supporting that Asp-394 of ANGase, that is Asp-647 of SPGase, is a catalytic residue.

Molecular Evolution of α-Glucosidase Families

The α-glucosidases belonging to family I have more unique properties than family II members. The enzyme (isozyme-II) from brewer's yeast attacks maltose and maltotriose, but shows no activity toward maltooligosaccharide larger than maltotetraose (5). Brewer's yeast isozyme-I (5) only hydrolyzes the α-1,6-linkage, and has no or less activity to other α-glucosidic linkages such as α-1,2-, α-1,3-, and α-1,4-bonds. An α-glucosidase from *Bacillus* sp. SAM1606 (24) showed the high activity to maltooligosaccharides. Even if there is the variety in substrate specificity, the preferred substrate of family I enzymes is a heterogeneous saccharide, such as aryl α-glucoside. This means that the recognition of α-glucosyl structure is very strict. There are two distinct types of hydrolases, dextrin 6-α-glucanohydrolase (EC 3.2.1.10, the dextran glucosidase is considered to belong to α-glucosidase) and trehalose-6-phosphate hydrolase (EC 3.2.1.93): the former produces glucose from dextran and its short chain oligosaccharides (25, 26) and the latter liberates glucose and glucose-6-phosphate from trehalose-6-phosphate (27). Both enzymes, which are unable to attack maltooligosaccharides, show the high hydrolytic activity toward aryl α-glucoside. Moreover, the primary structures of them are homologous

to that of family I α-glucosidase, implying that they have evolved from the same ancestral protein with keeping the structure to recognize α-glucosyl residue in substrate. This is a typical case of parallel evolution. The presence of two α-glucosidase families different in the substrate specificity, the whole amino acid sequence, and the catalytic site structures implies the convergent evolution that the distinct ancestral proteins have evolved and acquired the same catalytic reaction to hydrolyze the α-glucosidic linkage. α-Glucosidase is an "evolutional product" of convergence and parallelism.

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