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Dual biological role of glycoside hydrolase family fifty-seven glycogen branching enzymes

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**Dual biological role of glycoside hydrolase family
fifty-seven glycogen branching enzymes**

Gang Xiang

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Dual biological role of glycoside hydrolase family fifty-seven glycogen branching enzymes

PhD thesis

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and in accordance with
the decision by the College of Deans.

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Abbreviations

α -1,4 GT4: α -1,4 glucosyltransferase family 4

ACL: average chain length

AcT: acetyltransferase

ADPG: adenosine 5'-diphospho- α -d-glucopyranose

AF4: asymmetrical flow field flow fractionation

AGPase: glucose-1-phosphate adenylyltransferase

AM: amylomaltase

AmyC: GH57 GBE from *Thermotoga maritima* SMB8

AS: amylosucrose

ATP: adenosine 5'-triphosphate

BCA: bicinchoninic acid

BLAST: basic local alignment search tool

CBM48: carbohydrate-binding module family 48

CBP: cellobiose phosphorylase

CDC: coding sequence

CL: chain length

CLn: number-average chain length

CSR: conserved sequence region

D₂O: deuterium oxide

Da: dalton

DB: degree of branching

DGG: diglucosylglycerate

DggS: diglucosylglycerate synthase

DP: degree of polymerization

DPw: weigh-average degree of polymerization

DUF: domain of unknown function

ECL: exterior chain length

G1P: glucose-1-phosphate

G4: maltotetraose

GBE: glycogen branching enzyme

GG: glucosylglycerate

Abbreviations

GGBE: glucosylglycerate branching enzyme

GH13_8: GH13 subfamily 8

GH13_9: GH13 subfamily 9

GH13 GBE: glycogen branching enzyme from glycoside hydrolase family 13

GH57 GBE: glycogen branching enzyme from glycoside hydrolase family 57

GH: glycoside hydrolase

GlgE: maltosyltransferase

GlgM: maltose-1-phosphate synthase

GP: α -glucan phosphorylase

GPG: glucosyl-3-phosphoglycerate

GpgP: glucosyl-3-phosphoglycerate phosphatase

GpgS: glucosyl-3-phosphoglycerate synthase

GSE: glycogen synthase

GT: glucosyltransferase

HBM: highly branched maltodextrin

HBS: highly branched starch

^1H -NMR: proton nuclear magnetic resonance

HPAEC-PAD: high performance anion exchange chromatography coupled with pulsed amperometric detection

ICL: interior chain length

IM: isoamylose

IPTG: isopropyl β -D-1-thiogalactopyranoside

LB: Luria-Bertani medium

LDH: L-lactate dehydrogenase

M1P: maltose-1-phosphate

MalG: maltosylglycerate

MALLS: multi-angle laser light scattering

MalQ: α -1,4 glucanotransferase

MeT: methyltransferase

MGLP: methylglucose lipopolisaccharide

MMP: methylmannopyranose polysaccharide

Ms GH57 GBE: GH57 GBE from *Meiothermus* sp. PNK-Is4

Mt GH57 GBE: GH57 GBE from *Mycobacterium tuberculosis* H37Rv

M_w: weight-average molecular weight
MW: molecular weight
NADH: nicotinamide adenine dinucleotide
NCBI: national center for biotechnology information
PBS: phosphate buffered saline
PDB: protein data bank
Pep2: maltose kinase
3-PGA: 3-phosphoglycerate
Pi: phosphate
PK: pyruvate kinase
PmGgs: glucosylglycerate synthase from *Petrotoga mobilis* SJ95
Pm GSE: glycogen synthase from *Petrotoga mobilis* SJ95
PMPS: polymethylated polysaccharides
QELS: quasi-elastic light scattering
RDM: rapidly digestible maltodextrin
RDS: rapidly digestible starch
Rg: radius of gyration
Rm GBE: GH13 GBE from *Rhodothermus marinus*
RS: resistant starch
SAH: S-adenosylhomocysteine
SAM: S-adenosylmethionine
SAM-MT: S-adenosylmethionine-dependent methyltransferase
SDM: slowly digestible maltodextrin
SDS: slowly digestible starch
SEC: size exclusion chromatography
SP: sucrose phosphorylase
SuSy: sucrose synthase
TreS: trehalose synthase
Tt GH57 GBE: GH57 GBE from *Thermus thermophilus* HB8
UDP: uridine 5'-diphosphate
UDPG: uridine 5'-diphospho- α -d-glucopyranose
UPF: uncharacterized protein family

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Preface

Polysaccharides like proteins and polynucleotides are naturally occurring macromolecules present in living cells having structural, functional, or regulatory roles in biological processes. Polysaccharides are a group of diverse biopolymers with a myriad of sugar monomers and glycosidic linkages. Glycogen, a highly branched glucopyranose polysaccharide, is found in many organisms as energy reservoir and carbon store. Glucopyranose units in the backbone of glycogen are linked by α -1,4-*O*-glycosidic bonds and occasionally branched by α -1,6-*O*-glycosidic bonds. In eukaryotic cells, such as those of human and yeast, glycogen is biosynthesized by a simultaneous action of glycogen synthase (GSE), which is responsible for introducing α -1,4-*O*-glycosidic bonds, and glycogen branching enzyme (GBE), which creates branches attached via α -1,6-*O*-glycosidic bonds, with glycogenin as primer and uridine 5'-diphospho- α -d-glucopyranose (UDPG) as glycoside donor. Similarly, biosynthesis of glycogen is usually achieved by the above-mentioned classical metabolism with pre-existing oligosaccharides as primer in prokaryotic organisms. GBE plays an essential role in glycogen metabolism *in-vivo* by acting on growing α -glucans which are generated by GSE-catalyzed polymerization reaction. Due to capability of creating branches, GBEs are utilized to modify starches in starch-processing industry for the purpose of production of slowly digestible starch.

GBE catalyzes α -1,6-*O*-glycosidic linkage formation by cleaving an α -1,4-*O*-glycosidic bond and transferring the cleaved-off glucan to the same (intrachain branching) or a different glucan chain (interchain branching). Two distinct families, namely glycoside hydrolase (GH) family 13 and GH57, were classified among GBEs according to sequence similarity and motifs. GH13 GBEs carry a central $(\beta/\alpha)_8$ catalytic domain, an N2 β -sandwich domain (also annotated as carbohydrate-binding module family 48 or CBM48), and a C-terminal β -sandwich domain. In GH57 GBEs, a catalytic $(\beta/\alpha)_7$ barrel, a domain B, and an α -helix-rich C-terminal domain are found. Although the domains vary between GH13 GBEs and GH57 GBEs, they all possess two conserved acidic residues (aspartate and glutamic acid) as catalytic residues and employ a double-displacement reaction mechanism, resulting in the retention of the anomeric configuration of the glycosidic bond.

The occurrence of GH57 GBEs is widely noted in prokaryotic microorganisms, such as bacteria from the phylum Actinobacteria and Cyanobacteria, and archaea from the genus *Thermococcus*. The first member of GH57 GBEs to be biochemically characterized was the one from the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. Since then, four other GH57 GBEs (*Petrotoga mobilis* SJ95, *Pyrococcus horikoshii* OT3, *Thermotoga maritima* SMB8 and *Thermus thermophilus* HB8) from both bacteria and archaea have been studied. A straightforward association of the role of branching glycogen and the characterized glycogen branching activity in these five GH57 GBEs was conceived. However, this is not the case for all GH57 GBEs. For example, a peculiar GH57 GBE from *Mycobacterium tuberculosis* H37Rv, having all GH57 GBE key structural features, fails to display any branching activity on a wide range of α -glucans. Several studies indicate that this GH57 GBE possibly associates with the biosynthesis of methylglucose lipopolysaccharide (MGLP), an unusual mycobacterial intracellular polymethylated polysaccharide. However, this potential biological role remains unsolved with no solid experimental results available so far.

Outline of this thesis

As summarized here, GH57 GBEs are far less studied and understood than GH13 GBEs. Ambiguous physiological roles of GH57 GBEs can be proposed from various available studies. In addition, limited knowledge concerning substrate specificity of GH57 GBEs is available. Previously published biochemical characterization studies showed that the behaviour of GH57 GBEs towards amylose with respect to activities and product properties is clearly different from that of GH13 GBEs. This contributes to a limited understanding of the physiological role of GH57 GBEs, in particular for those microorganisms in which GH13 GBE and GH57 GBE are both present. To shed some light on the substrate specificities and biological roles of GH57 GBEs, an extensive investigation including bioinformatics and in-depth biochemical characterization of a number of selected GH57 GBEs was performed. The results of these studies are included in this thesis.

This thesis contains one review chapter, four experimental chapters, one summary and one future perspective chapter:

Chapter 1: General introduction.

This chapter presents a state-of-the-art literature review of the carbohydrate active enzyme families glycoside hydrolases (GHs) and glycosyltransferases (GTs), and of the two polysaccharides: glycogen and MGLP, including the *in-vivo* metabolism of these polysaccharides and the role of GBEs in the biosynthesis of glycogen.

Chapter 2: Structural elements determining the transglycosylating activity of glycoside hydrolase family 57 glycogen branching enzymes.

This chapter aims at acquiring a better understanding of the transglycosylating activity of GH57 GBEs by a systematic sequence-based bioinformatics and biochemical characterization analysis. Sequence-based bioinformatics showed that two GH57 GBE-specific sequence logos, binary variations (presence/absence) in flexible loop and two β -strands were noted. A correlation was found between branching activity of GH57 GBEs and structural elements including a flexible loop, a tyrosine at the loop tip, and two β -stands. In conclusion, bioinformatics analysis and biochemical data showed that putative GH57 GBE sequences can be defined by GH57 GBE-specific sequence logos and true GH57 GBEs can be designated by optimal length of flexible loop and the presence of two β -strands.

Chapter 3: The *Mycobacterium tuberculosis* H37Rv GH57-type glycogen branching enzyme homologue Rv3031 is involved in methylglucose lipopolysaccharide biosynthesis.

The purpose of this chapter is to solve the key step of the conversion of glucosylglycerate (GG) to diglucosylglycerate (DGG) in the biosynthesis of MGLP. This step is possibly catalyzed by a GBE from GH57 according to previous studies. Genomic analysis showed that a conserved gene cluster of (*sam-mt*)-GH57 GBE-*gt4-act* exists exclusively in the phylum Actinobacteria, including all *Mycobacterium* species. This conserved cluster presents an indication of a potential role of GH57 GBE in the MGLP biosynthesis. Experiments with recombinant *Mycobacterium tuberculosis* GH57 GBE (Mt GH57 GBE) were conducted to investigate the aforementioned conversion. A range of potential glycoside donors and GG were offered to the recombinant Mt GH57 GBE. HPEAC-PAD data showed that an extra peak was solely identified using maltotriitol as glycoside donor, suggesting that an unknown product was derived from GG and maltotriitol by Mt GH57 GBE. Our data suggest that although being homologous to GH57 GBEs, the *M.*

tuberculosis Rv3031 is not involved in branching glycogen but is a glucosylglycerate branching enzyme involved in the biosynthesis of MGLP.

Chapter 4: The intrinsic properties of GH57 GBEs control performance towards growing α -glucans.

This chapter deals with the *de-novo* synthesis of α -glucans *in-vitro* by creating conditions resembling the *in-vivo* situation to some extent. This was achieved by a combined action of GSE with a GH57 GBE, and allowed the investigation of the activities of GH57 GBEs and the nature of products towards growing α -glucans. α -Glucan synthesis was conducted by combining the *Petrotoga mobilis* GSE and one of three GH57 GBEs (*Thermus thermophilus*; Tt, *Meiothermus* sp; Ms and *Mycobacterium tuberculosis*; Mt) with a catalytic activity ratio of 5:1 from maltotetraose and UDPG in a ratio of 1:500. Catalytic activities and structural features including degree of branching and molecular weight distribution were studied. The results show that catalytic activities of Tt GH57 GBE and Ms GH57 GBE on a growing α -glucan chain are comparable to those found when using amylose as substrate. No activities were found for the Mt GH57 GBE. Molecular weight distribution and degree of branching data show that branched α -glucans were obtained in Tt GH57 GBE or Ms GH57 GBE in combination with Pm GSE. In contrast, only linear α -glucans were produced when the Mt GH57 GBE was coupled to the Pm GSE.

Chapter 5: Amylomaltase raises the maximum degree of branching reached by glycogen branching enzymes in starch modification.

Chapter 5 focuses on application aspect of GBE, converting gelatinized tapioca starch into highly branched maltodextrins (HBM) with a reduced digestibility. Oligosaccharide by-products were formed due to the hydrolytic activity of GBEs, thereby reducing the overall efficiency. Amylomaltase (AM) catalyzes disproportionation from oligosaccharides into shorter and longer chains. The combined action of the *Thermus aquaticus* AM with the *Rhodothermus marinus* (GH13) or *Thermus thermophilus* (GH57) GBE was applied to reduce average degree of polymerization of the oligosaccharide by-products. The results show that AM in combination with GBE enhances the conversion efficiency of starch into HBM. Moreover, the dual enzymatic conversion yielded maltodextrins with a significantly higher degree of branching (in the case of Tt GH57 GBE) and a considerably reduced *in-vitro* digestion rate than that of only GBE treated gelatinized tapioca starch.

Chapter 6 and 7: Summary and future perspectives.

Chapter 6 summarizes main results and conclusions drawn from this thesis. The dual substrate specificity of GH57 GBEs is governed by binary variations in the flexible loop and two β -strands. The dual distinct substrate specificity potentially poses dual biological role of GH57 GBEs. Future perspectives on GH57 GBEs were also included with several proposals for future research on GH57 GBEs to understand the structural features and dual biological role in more detail.

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