Smriti Shrivastava *Editor*

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To My Family and Research Group

Foreword

Glycoside hydrolases (glycosidases or glycosyl hydrolases) are specific catalysts involved in breaking down glycosidic bonds of complex polysaccharides, such as cellulose, hemicelluloses, and starch. The cellulases, amylases, xylanases, and arabinases are examples of few enzymes of the group, and complete classification data of these enzymes describe their diverse mode of action, making them capable of acting on varied substrate range and thus breaking down the complex molecules to simple components that can be promisingly used for the production of a wide range of value-added products. It is this feature of the enzymes which makes them highly industrially significant. Alongside they also function as antibacterial defense proteins and help in normal cellular function and pathogenesis in the case of viruses. These enzymes have been studied since the last 60–70 years and, though being highly utilized in industries, still find numerous applications and high scope for research. This work has been designed focusing on industrial applications of glycoside hydrolases and covers reports on recent tools and techniques for enhancing enzyme performance.

Since they are numerous in number and found in diverse cells, a comparative analysis of the same will give a clear picture of the utilization of these enzymes in various industrial targets, benefiting the large research group working on glycoside hydrolases. With this concept in cognizance, chapter 1 describes a formatted detail on all glycoside hydrolases with specific characteristics in their three-dimensional structures, thus enabling their classification into 166 families that can again be grouped under 16 superfamilies. After considering the base classification of glycoside hydrolases, an understanding of structure and applications of glycoside hdyrolases is highly essential and has been brought forward as chapter 2, with a

note on recent tools and synergistic effect of enzymes for improving catalytic activity depicted in chapters 3 and 9.

This book features industrial applications of glycoside hydrolases, which have been enumerated well, in paper and pulp industries, bioenergy segment, and food industries, and the use of marine glycoside hydrolases (described in chapters 5–8). Glycoside hdyrolases find major applications in these industrial segments. The last section of the book covers functional and comparative genomic analysis of glycoside hydrolases and gives a description of metagenomics and its application for studying cellulases (Chapter 10).

It is anticipated that this book will be an excellent knowledge source for different sections of the scientific community to understand the functioning of diverse glycoside hydrolases and further exploring the promising applications of these enzymes.

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(Pratyoosh Shukla)

Preface

Energy and environmental security are two major concerns of the present world. We at our laboratory are working on the production of value-added components through enzymatic hydrolysis of Indian agricultural residues. In the process, I came across a few hydrolytic enzymes (belonging to glycoside hydrolases), responsible for saccharification of polysaccharides (hemicelluloses), and subsequent reading on the subject improved my understanding on the wide utility of glycoside hydrolases and its application in numerous industries, thus initiating work on compiling details of glycoside hydrolases with its major applications.

Glycoside hydrolases are resourceful tools in glycobiology and an important catalyst for industrial and biotechnological processes. They have a high level of structural diversity and due to their exquisite specificity and excellent catalytic efficiency, they find a wide range of distinct applications from biomass degradation to cell surface engineering.

This volume summarizes and updates both the state of knowledge and theories on structure, function, and biotechnological applications of glycoside hydrolases. It will be of great interest to diverse research groups working on glycoside hydrolases, in particular to the group working on industrial applications of these enzymes.

While planning this book, invitations for contributors were extended to subject experts. I would like to express my deep appreciation to each contributor for their patience and attention during the production process. This book contains 10 chapters, grouped under 3 sections that include (A). Introduction, (B). Industrial Applications, and (C). Functional and Comparative Genomic Analysis of Glycoside Hydrolases.

I am extremely delighted to edit this volume, due to the stimulating cooperation of the contributors. I wish to generously thank Dr. Bhavik Shawney and Mr. Lenold Christ Raj, Springer Nature, SPi Global for their munificent assistance in finalizing the volume. My special thanks to family, friends, colleagues, and students and sincere thanks to all contributors.

Noida, India — Smriti Shrivastava

Acknowledgments

I would like to express my sincere and heartfelt thanks to my family, for their constant support throughout my career. My special thanks to Prof. Ashok Chauhan (Founder President, Amity University) for giving us an excellent platform to work and thanks to each member of the Amity University Uttar Pradesh, Noida. I would certainly not have been able to compile this book without the wonderful contributions of each author, who has excellently given their inputs. My sincere thanks to all the authors for sharing their knowledge and expertise through this book. My special thanks of gratitude to all my mentors throughout my journey to build me day by day. My earnest thanks to the editorial team, Springer to help in this journey all the way through. And last of all, I would be extremely thankful to all readers of my book and believe that this book will prove to be of high interest and significance to them.

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Abbreviations

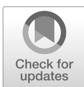
AD	Anaerobic digestion
ADH	Alcohol dehydrogenase
AFEX	Ammonia fiber expansion
AGX	Arabinoglucuronoxylans
AI	Arabinose isomerase
AR	Arabinose reductase
AXs	Arabinoxylans
BOD	Biological oxygen demand
CAGR	Compound annual rate of growth
CAZy	Carbohydrate-Active EnZymes
CBD	Cellulose binding domain
CBM/CBD	Carbohydrate binding module/domain
CBP	Consolidated bioprocessing
CDH	Cellobiose dehydrogenase
CE	Carbohydrate esterase
CHS	Chitosan
CMC	Carboxymethyl cellulase
COS	Chitooligosaccharides
DIMBOA	2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one
DP	Degree of polymerization
EC	Enzyme Commission
EMP	Embden–Meyerhof–Parnas
Eno	Enolase
EPS	Extracellular polymeric substances
ERA-NET (ERA-MBT)	European Marine Biotechnology
FA	Fructose bisphosphate aldolase
FAD	Flavin adenine dinucleotide
FCR	Feed conversion rates
FE	Feruloyl esterase
FOS	Fructooligosaccharides
GAXs	Glucuronoarabinoxylans
GDH	3-phosphate dehydrogenase
GH	Glycoside hydrolases

GlcNAc	*N*-acetyl glucosaminidases
GOS	Galactooligosaccharides
GPDH	Glucose-6-phosphate dehydrogenase
GT	Glycosyltransferase
GXs	Glucuronoxylans
HEWL	Hen egg-white lysozyme
HK	Hexokinases
HMF	Hydroxymethyl furfural
IUBMB	International Union of Biochemistry and Molecular Biology
KDNase	*Aspergillus fumigatus* Sialidase
KDPG	2-keto-3-deoxy-6-phosphogluconate
KDPGA	2-keto-3-deoxy-6-phosphogluconate aldolase
Lac	Lactonase
LAD	L-arabitol dehydrogenase
LPMO	Lytic polysaccharide mono-oxygenase
L-XuR	L-xylulose reductase
MLF	Malolactic fermentation
MOS	Mannooligosaccharides
NAD	Nicotinamide adenine dinucleotide
NAD(H)	Nicotinamide adenine dinucleotide (reduced form of NAD)
NAG	N-acetyl-D-glucosamine
NAM	N-acetylmuramic acid
NSPs	Non-starch polysaccharides
O-GlcNAcase	(protein)-3-*O*-(*N*-acetyl-D-glucosaminyl)-L-serine/threonine *N*-acetylglucosaminyl hydrolase
PDB	Protein Data Bank
PDC	Pyruvate decarboxylase
PFK	Phosphofructokinase
PGD	6-phosphogluconate dehydratase
PGK	Phosphoglycerate kinase
PGM	Phosphoglycerate mutase
PI	Phosphoglucose isomerase
PK	Pyruvate kinase
PKL	Phosphoketolase
PL	Polysaccharide lyase
POS	Pectin oligosaccharides
PPE	Phosphopentose epimerase
PPP	Pentose phosphate pathway
PUGNAc	O-(2-Acetamido-2-deoxy-D-glucopyranosylidenamino) N-phenylcarbamate
R5PE	Ribulose-5-phosphate-4-epimerase
RK	Ribulokinase
SSCF	Simultaneous saccharification and co-fermentation

SSF	Solid state fermentation
TA	Transaldolase
TIM	Triosephosphateisomerase
TK	Transketolase
TPI	Triosephosphate isomerase
VDECH	Endochitinase from *Verticillium dahlia*
XDH	Xylitol dehydrogenase
XI	Xylose isomerase
XK	Xylulose kinase
XOS	Xylooligosaccharides
XR	D-xylose reductase

Part I

Introduction



Introduction to Glycoside Hydrolases: Classification, Identification and Occurrence

Smriti Shrivastava

Abstract

Glycoside hydrolases are group of enzymes belonging to class 3 enzymes as per classification by IUBMB. They specifically break down glycosidic bonds of complex polysaccharides and are generally named upon the substrates on which they act (e.g. lactase acting on lactose; chitinase acting on chitin, sucrase acting on sucrose, etc). Present chapter introduces glycoside hydrolases, its identification and occurrence in nature, highlighting the diverse existence of these enzymes. It covers a detailed report on classification and available three-dimensional structures of the enzymes of the group. Glycoside hydrolases have been classified under 166 families which are grouped in 16 different superfamilies and three added clans. The chapter gives a complete documentation of all available data on glycoside hydrolases, which will be beneficial for researchers working in the domain.

Keywords

Glycoside hydrolases · Enzyme classification · Enzyme nomenclature · Glycoside hydrolase superfamilies

Introduction

Glycoside hydrolases (Glycosidase or glycosyl hydrolases) are specific catalyst that breaks down glycosidic bonds of complex polysaccharides, such as cellulose, hemicelluloses, starch etc. These enzymes with their specific functions are called cellulase, amylases, xylanases, arabinases and includes several others. In addition to

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degradation of plant polysaccharides they also function in anti bacterial defense mechanism (lysozymes), in normal cellular functioning (such as biosynthesis of N-linked glycoprotein through mannosidases), pathogenesis through viral neuraminidase (Bourne and Henrisatt 2001; Henrisatt and Davies 1997)

Occurrence and Importance

Present in almost all domains of life, glycoside hydrolases are found both as intracellular and extracellular enzymes in prokaryotes and are majorly involved in hydrolysis of glycoside molecules, nutrient acquisition, regulation of expression of operon, post translational modification, lysosomal storage in higher organisms, biosynthesis and degradation of glycogen. In prokaryotes and lower eukaryotes they are present intracellular as well as secreted as extracellular enzymes. In higher organisms they are found within endoplasmic reticulum and golgi apparatus (processing of N-linked glycoproteins); in lysosome (degradation of carbohydrate structure) in intestinal tract and in saliva as carbohydrate degraders (amylase), in gut as glycosylphosphatidyl anchored enzymes on endothelial cells as enzyme lactase (degradation of milk sugar lactose), enzyme O-GlcNAcase (removal of N-acetylglucosamine groups from cytoplasmic and nuclear located serine and threonine residues)

Mechanisms of Glycoside Hydrolases

Basis their mechanism of action, Glycoside hydrolases are broadly classified as inverting glycoside hydrolases and retaining glycoside hydrolases.

Inverting enzymes utilize two enzymic residues (generally carboxylate), one act as acid and other as base. Figure 1 shows mechanism of action of a β-glucosidase.

Fig. 1 Mechanism of action of a β-glucosidase

Fig. 2 Mechanism of action for hen egg white lysozyme

Fig. 3 Mechanism of action of N-acetyle Hexoaminidases

Retaining glycoside hydrolases conduct hydrolysis through two main mechanisms, with each step resulting in inversion. Two residues involved are generally enzyme accepted carboxylates; of which one acts as a nucleophile and the other as acid/base. First step involves attack of nucleophile to the anomeric center forming a glycosyl enzyme intermediate (acidic assistance provided by acid carboxylate), followed by hydrolysis of glycosyl-enzyme transitional state through nucleophilic water (assisted by deprotonated acidic carboxylate, that acts as a base). This process results in net retention of stereochemistry. Mechanism has been illustrated as example for hen egg white lysozyme (Fig. 2) (Vocadlo et al. 2001).

Enzyme mechanisms that carried out hydrolysis with retention of stereochemistry occurs through a substrate bound nucleophilic residue, rather than being directly attached to the enzyme. Such mechanism can be seen for certain N-acetylhexosaminidases (Fig. 3), where an acetamido group present on the enzyme can participate with neighboring group and forms and intermediate oxazoline or oxazolinium ion following two steps mechanism of distinct inversions making net retention of configuration.

Applications of Glycoside Hydrolases

Glycoside hydrolases are one of the major catalysts of ensuing generation owing to its varied applications in biorefining processes. These includes hydrolysis of plant materials (cellulases, xylanases) for production of value added components,

applications in food industries (invertase for production of invert sugars; amylase for production of maltodextrins), usage in paper and pulp industry, in detergent manufacturing (cellualses for washing of cotton fabrics for maintenance of fabric colour by removing microfibre) (Linares-Pastén et al. 2014).

These enzymes are used as synthetic catalysts (performing reverse hydrolysis/ transglycosylation); reversing equilibrium and enabling the retaining glycoside hydrolase to catalyze transfer of glycosyl moiety from an activated glycoside to an acceptor alcohol. Glycosynthases (formed from retaining glycoside hydrolases by site directed mutagenesis of enzymic nucleophile to some less nucleophilic group like alanine/glycine) are enzymes catalyzing high yield of glycosides from activated glycosyl donors like gycosyl fluorides. Thioglycoligases also are mutant glycoside hydrolases, formed by site directed mutagenesis of the acid base residue of retaining glycoside hydrolases and catalyze condensation of activated glycosides and thiol comprising acceptors.

Glycoside hydrolases show usefulness in matrix polysaccharide within extracellular polymeric substances (EPS) of microbial biofilm (Fleming and Rumbaugh 2017). Degrading microbial biofilm, increases antibiotic efficacy, potentiating host immune function (Fleming et al. 2017).

Inhibitors if Glycoside Hydrolases

There are various natural and synthetic compounds that have been reported to act as inhibitors of glycoside hydrolases. This includes naturally occurring nitrogen containing sugar shaped heterocycles (deoxynojirimycin, swainsonine, australine, castanospermine) working as natural templates for developing modified inhibitors (e.g. isofagomine, deoxygalactonojirimycin, unsaturated compounds such as PUGNAc). Glycoside hydrolase inhibitors finding clinical usage includes anti-diabetic drugs (acarbose and miglitol) and antiviral drugs (oseltamivir and zanamivir). Few proteins have also been identified as Glycoside hydrolase inhibitors.

Classification of Glycoside Hydrolases

According to enzyme nomenclature by International Union of Biochemistry and Molecular Biology (IUBMB), Glycoside hydrolases are classified into EC 3.2.1 as enzymes catalyzing the hydrolysis of O- or S-glycosides (Sinnott 1990). They are also classified based on stereochemical outcome of hydrolysis reaction (retaining or inverting enyzmes); on exo (non-reducing end) and endo (middle of molecule) acting, on sequence and structure based classification. Sequence based classification has suggested more than 150 different families of glycoside hydrolases (Henrissat et al. 1995; Henrissat and Davies 1995; Bairoch 1999), these are available on CAZy Carbohydrate-Active Enzymes web site, supported by CAZypedia [Cazy family Glycoside hydrolase; Cazypedia; Henrissat and Coutinho 1999]. Sequence based

classification significantly helps in prediction of mechanism of action of enzyme, active site residues and possible substrates. Enzymes further classified as clans of related structure based on three dimensional structural similarities obtained from available sequences (Naumoff 2006, 2011).

Glycoside hydrolases (O-Glycosyl hydrolases) EC 3.2.1.X catalyzing hydrolysis of glycosidic bond are classified based on sequence similarity to be most reliable and led to the definition of 128 families and 14 clans (based on folds of proteins) of the same (Henrissat et al. 1995; Henrissat and Davies 1995; Henrissat and Bairoch 1996). This sequence based classification is available on the CAZy (Carbohydrate-Active Enzymes) (Cantarel et al. 2009). Glycoside hydrolases are also classified based on their localization in cell (secreted, monotopic, peripheral, lysosomal, located in eukaryotic plasma, inner membrane of Gram positive bacteria).

Following section in the chapter will deal with the major features and mode of action of all glycoside hydrolases classified. One prominent known structure from each family is depicted in Fig. 4a-r5.

Glycoside Hydrolase Family 1

Enzymes of family 1 follow the IUBMB EC 3.2.1 nomenclature and catylzes hydrolysis of glycosidic bond between two carbohydrate molecules or a carbohydrate and non carbohydrate molecule (Lombard et al. 2014; Cazypedia.org and Cazypedia Consortium). Major enzymes of Glycoside Hydrolase family 1 are; beta-glucosidase (EC 3.2.1.21); beta-galactosidase (EC 3.2.1.23); 6-phospho-beta-galactosidase (EC 3.2.1.85); 6-phospho-beta glucosidase (EC 3.2.1.86); lactase-phlorizin hydrolase (EC 3.2.1.62), lactase (EC 3.2.1.108); beta mannosidase (EC 3.2.1.25); myrosinase (EC 3.2.1.147). Figure depicts general structure of Glycoside hydrolase family 1. According to the PROSITE documentation (https://prosite.expasy.org/cgi-bin/prosite/prosite-search-ac?PDOC00495#description) (Henrissat 1991a, b; Gonzalez-Candelas et al. 1990; El Hassouni et al. 1992) classifies β-glucosidases (EC 3.2.1.21); β-Galactosidases (EC 3.2.1.23); 6-phospho-β-Galactosidases (EC 3.2.1.85); β-Glucosidases (EC 3.2.1.86); plant myroniases (EC 3.2.1.147; e g. synigrinases or thioglucosidases); Mammalian lactase-phlorizin hydrolase (LPH; EC 3.2.1108/EC 3.2.1.62) in family 1 of Glycoside hydrolases. Conserved regions are central glutamic acid residues, acting as nucleophile during glycosidic bond cleavage. This is marked as signature pattern for this group of enzymes with another along with a conserved region containing glutamic acid found at their N-terminal extremity (Withers et al. 1990). This group belongs to the TIM Barrel glycoside hydrolase superfamily contains the range of enzymes belonging to group that posses a TIM barrel fold merging clans GH-A, GH-D, GH_H and GH-K. It contains 57 families and 259,156 domains and was built by A Bateman (Naumov and Karreras 2009). All members of family 1 glycoside hydrolases for which localization is known are typically restricted to plasma membrane and endoplasmic reticulum membrane (Davies and Henrissat 1995; Henrissat et al. 1995) (Fig. 4a).

Glycoside Hydrolase Family 2

Glycoside hydrolases family 2 enzymes comprises enzymes with β-galactosidases (EC 3.2.1.23); β-mannosidases (EC 3.2.1.25), β-glucuronidase (EC 3.2.1.31). Glutamic acid residue is the general acid/base catalyst at active sites of these enzymes (Gebler et al. 1992). The catalytic domain of β-galactosidases contains TIM barrel core surrounded beta domains, with sugar binding domain forming jelly–roll fold, containing immunoglobulin like beta-sandwich domain (Jacobson et al. 1994). General structure of glycoside hydrolase family 2 enzymes is elaborated as its

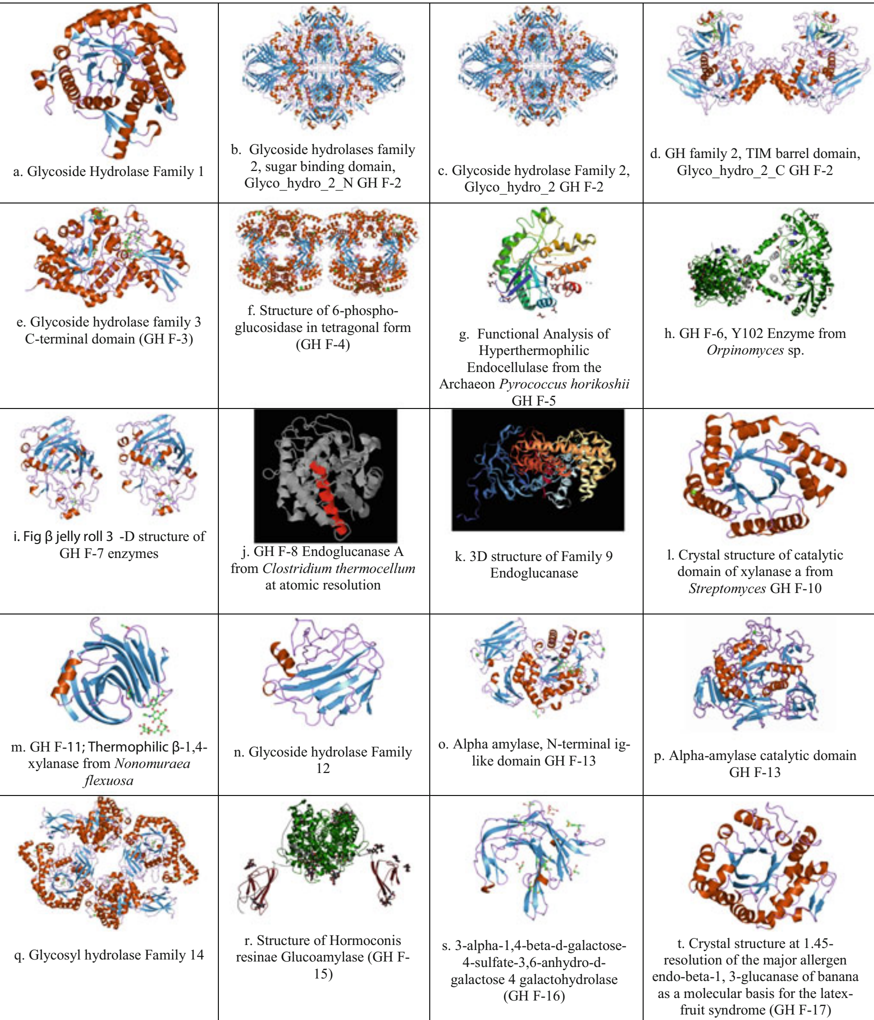


Fig. 4 Glycoside hydrolase family 1-Family 166 (One PDB accession for each family)

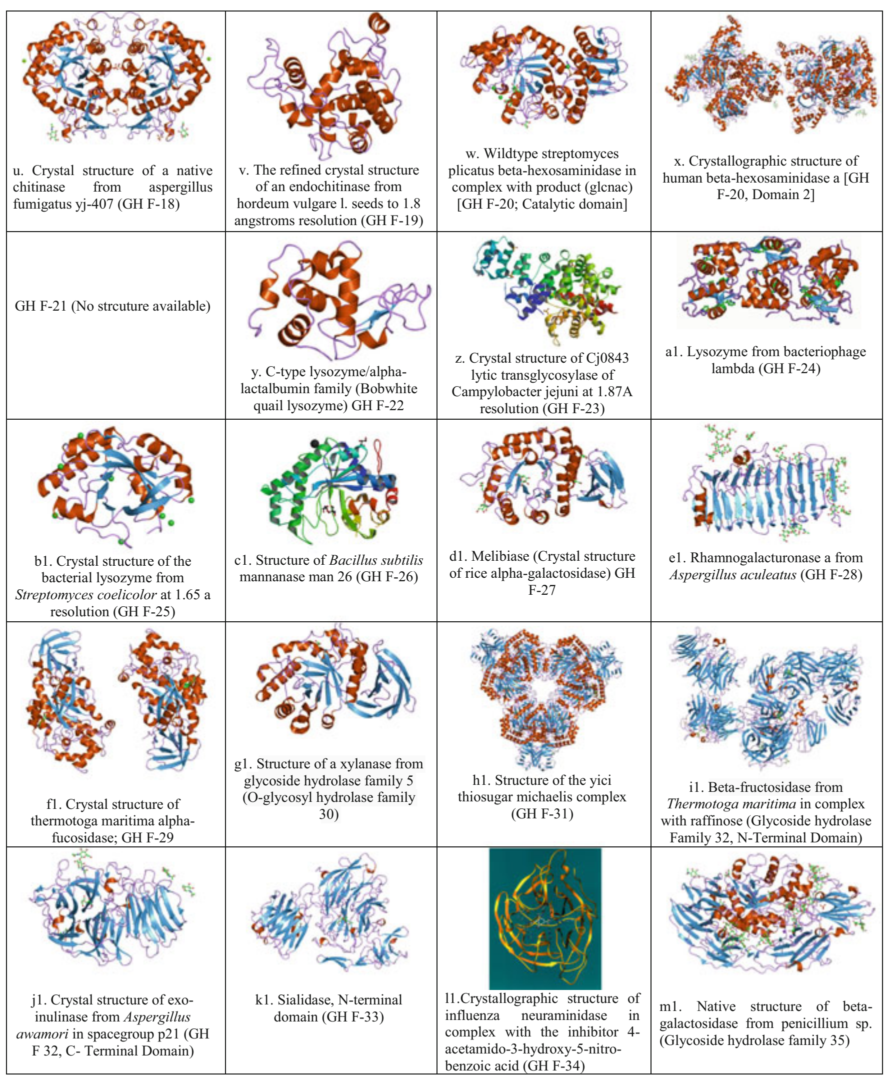


Fig. 4 (continued)

complete picture, sugar binding domain and TIM Barrel domain as depicted in Fig. 4b–d respectively.

Sugar binding domain belongs to the galactose binding domain like superfamily, the clan that contains 70 families and 124,105 domains. This superfamily has prominent sandwich domains with a jelly roll topology and is mainly involved in

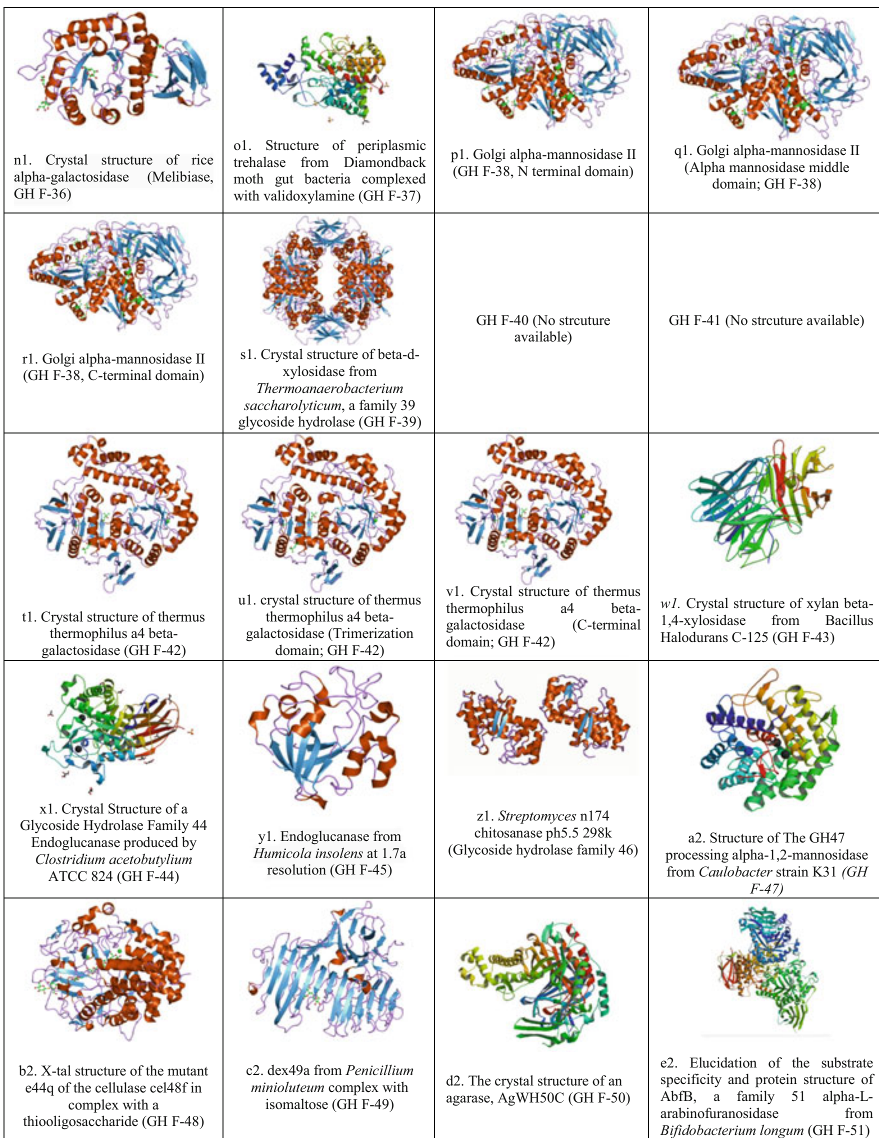
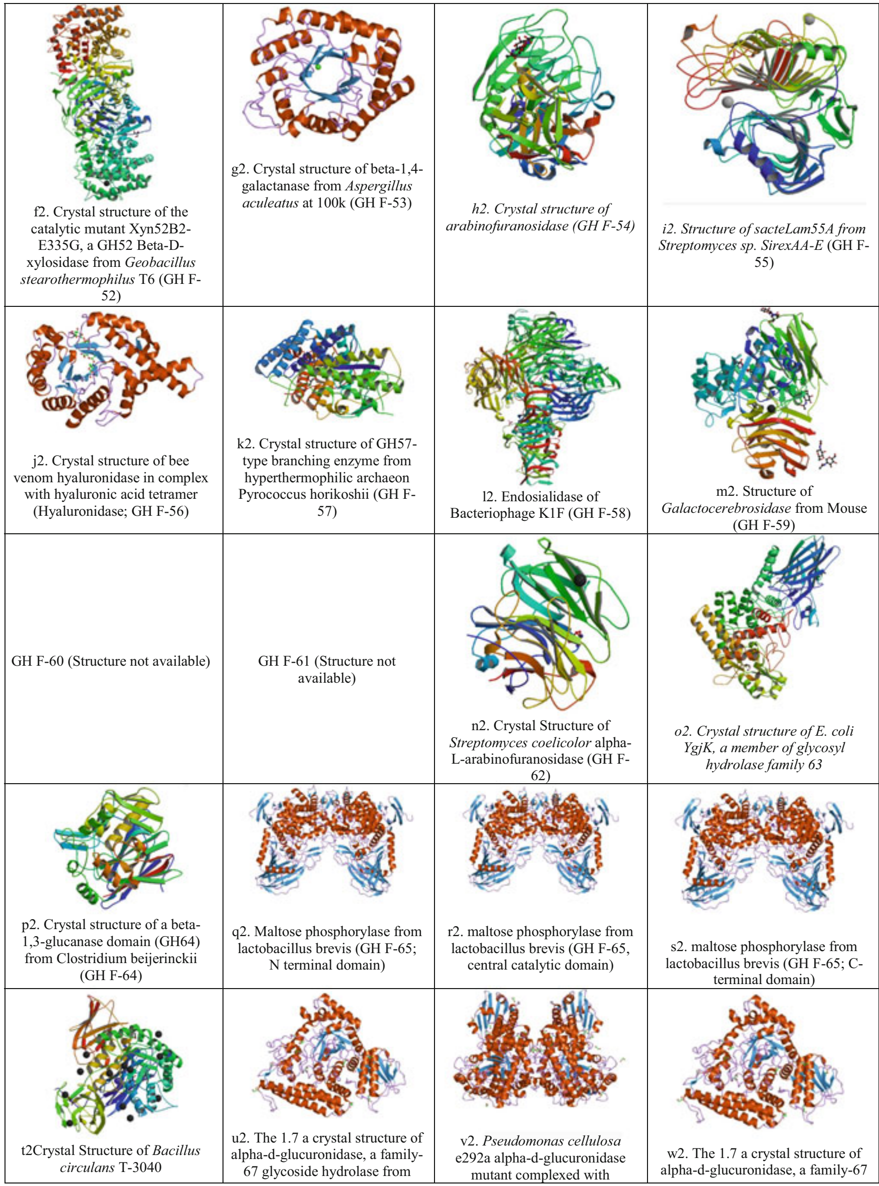


Fig. 4 (continued)

carbohydrate recognition. They share very little sequence similarity and weak sequence motif with conserved bulge (possibly helps in bending of beta sheets) in the C-terminal beta sheet, enabling curvature of sheet that forms a sugar binding site (Murzin and Bateman 1998).



f2. Crystal structure of the catalytic mutant Xyn52B2-E335G, a GH52 Beta-D-xylosidase from *Geobacillus stearothermophilus* T6 (GH F-52)

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r2. maltose phosphorylase from lactobacillus brevis (GH F-65, central catalytic domain)

s2. maltose phosphorylase from lactobacillus brevis (GH F-65; C-terminal domain)

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w2. The 1.7 a crystal structure of alpha-d-glucuronidase, a family-67

Fig. 4 (continued)

Glycoside Hydrolase Family 3

Glycoside hydrolase family 3 enzymes are two domain globular proteins N-glycosylated at 3 sites (Varghese et al. 1999). This family comprises of