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Chapter 6

Glycosidases – A Mechanistic Overview

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1. Introduction

Carbohydrates are the most abundant and structurally diverse class of biological compounds in nature. However, our current understanding regarding the relationship between carbohydrate structure and its biological function is still far from what is known regarding proteins and nucleic acids. Initially, carbohydrates were only recognized as structural and energy storage molecules (e.g. cellulose, chitin and glycogen), but recent developments in the field have shown that carbohydrates are also involved in numerous biological events, such as cancer, inflammations, pathogen infections, cell-to-cell communication, etc. In addition, carbohydrate-processing enzymes have become the choice in many industrial applications due to their stereo-selectivity and efficiency.

Carbohydrates can be found in nature in many forms, from simple monomers to more complex oligomers, polymers or glicoconjugates. The complexity of these structures can be reasonably high since each carbohydrate monomer can accommodate multiple linkages and/or branches in its structure. Moreover, as the glycosidic linkage between each monosaccharide can have two anomeric configurations (α or β), even in small oligosaccharides, the potential number of structures that can exist is huge.

In the last decades, there has been a great effort to synthesize oligo- and poly-saccharides in the laboratory, mainly due to their key role in many biological events but also to the interest expressed by the food and technical industries. The chemical approaches to carbohydrate synthesis have been known since Arthur Michael first reported the synthesis of a natural glycoside in 1879. However, the construction of complex carbohydrates and glycoconjugates in the laboratory remains a challenging endeavor. The causes for these difficulties are several but they mainly rely on the exceptional complexity and diversity that some compounds may show. Indeed, unlike the systematic processes of proteins and nucleic acids synthesis, in which the order of attachment of amino acids and nucleotides is read...
from a nucleic acid matrix, the synthesis of carbohydrates is a non-template-directed process that is controlled by a complex stereo- and regio-specific process. It requires a special regioselective reaction at a particular position of the sugar unit, in which the hydroxyl group that is available in such position must be distinguished from all the other hydroxyl groups in the structure that have similar properties. Additionally, the linkage between sugars must proceed through a stereoselective manner, since the linkage can produce two stereoisomers and one of them must be preferred to the other. Many carbohydrates are also found linked to protein and lipids. The synthesis of glycoconjugates has also proven to be a difficult task because it generally involves the participation of multiple transporters and enzymes. The mechanisms governing the regulation of these pathways are still being elucidated, but so far it has been found that the assembly of carbohydrates to proteins and lipids requires a specific chemistry that is far from being universal.

The production of oligosaccharides and polysaccharides has been deeply studied in the past decades and revealed to be, as expected, a challenging task. In spite of the advances observed in organic chemistry, the chemical synthetic routes addressed to synthesize these compounds have proven to be inefficient in the majority of the cases. This happens because the preparation of complex oligosaccharides and polysaccharides require multiple protection/deprotection and purification steps, which often lead to a tedious and time-consuming process and normally result in poor yields. To overcome these limitations, the enzymatic synthesis rapidly gained more prominence. The attractiveness of enzymatic synthesis is that protecting groups are not required and the stereo- and regio-selectivity chemistry is always followed in the formation of the glycosidic linkages, in the majority of the cases.

Enzymatic formation and cleavage of the bond between two sugars or between a sugar and another group can occur by hydrolysis to give the free sugar (glycosidases), by transglycosylation to give a new glycoside (glycosyltransferases), by phosphorolysis to give the sugar-1-phosphate (phosphorylases) or by elimination to give unsaturated sugar products (lyases). Currently, glycosidases and glycosyltransferases are the major classes of biocatalysts that are available for the enzymatic synthesis of polysaccharides and oligosaccharides. As the structure of lysozyme was first solved in 1965, glycosidases have long been the subject of structural biology studies in order to understand the molecular details of substrate recognition and of catalysis. As a result, about three quarters of the 113 known families of glycosidases have at least a structural representative. In contrast, progress in the structural biology of glycosyltransferases has been slower. Part of the success in characterizing glycosidases is due to the high stability of these enzymes when compared with the glycosyltransferases and because they are very easy to isolate, being generally available from natural sources like seeds, micro-organisms or fungal cultures, as well as in higher organisms (typically plant seed, mollusks, etc). These facts have turned glycosidases into an attractive target for many industries involved in the food, the paper and pulp industry, as well as in organic chemistry, where glycosidases have proven to be extremely efficient catalysts, being capable of hydrolyzing the very stable glycosidic bonds in glycoconjugates, oligo- and poly-saccharides. The importance of glycosidases has also
attracted the attention of many pharmaceutical industries since they are involved in many biological processes such as cell-cell or cell-virus recognition, immune responses, cell growth, and viral and parasitic infections. Currently, they have been associated with many diseases, which result from the lack or dysfunction of a glycosidase and are used in the treatment of metabolic disorders, viral infections and even cancer.

Despite the current advances in the field and the exponential interest in glycosidases, many aspects of the mechanism of action of these enzymes remain hidden in the available experimental data, in particularly in the X-ray structures that figure in the protein databank. Taking this into account, we focus in this review in the current literature regarding the catalytic mechanisms of glycosidases.

2. Catalytic mechanism of glycosidases

Glycosidases (GH) are present in almost all living organisms (exceptions are some Archaeans and a few unicellular parasitic eukaryotes\(^7,8\)) where they play diverse and different roles. Taking into account the diversity of reactions that they catalyze as well as amino acid sequence and folding, glycosidases have been classified in many different ways. According to The IUBMB (International Union of Biochemistry and Molecular Biology) glycosidases are classified based on their substrate specificity and/or their molecular mechanism.\(^9,10\) However, this classification is far from gaining consensus. A necessary consequence of the EC classification scheme is that codes can be applied only to enzymes for which a function has been biochemically identified. Additionally, certain enzymes can catalyse reactions that fall in more than one class, which makes them bear more than one EC number. Furthermore, this classification does not reflect the structural features and evolutionary relations of enzymes. In order to overcome these limitations, a new type of classification was proposed based on the amino acid similarity within the protein. This new classification is available at the Carbohydrate-Active Enzymes database (CAZy - http://www.cazy.org/)\(^7,10\) and provides a direct relationship between sequence and folding similarities, that can be found in 130 amino acid sequence-based families. Some families with apparently unrelated sequence similarities show some uniformity in their three-dimensional structures. In those cases, these structures have been assigned to the so-called “Clans”, that have been numbered from A to N.\(^7\) In general, GHs belonging to the families of the same clan have common ancestry, similar 3D structure and are characterized by an identical catalytic mechanism of action.\(^7,10-16\)

The two most commonly employed mechanisms used by glycosidases to achieve glycosidic bond cleavage with overall inversion or retention of anomeric stereochemistry are shown schematically at Figure 1. These mechanisms can be generally divided in two main groups: the retaining GHs and the inverting GHs.\(^17,18\) Generally, enzymes of the same family have the same mechanism (but not specificity) \([41, 43, 63]\), and the only exception are the GH23 and GH97 families that combine retaining and inverting GHs.\(^19\) Table 1 summarizes the information about all GHs discovered until now.
Table 1. Organization of glycosidases families in clans and their correlation with the type of mechanism that they catalyze, and their protein folding.

### 2.1. Retaining GHs

The catalytic mechanism of retaining glycosidases was proposed about 58 years ago by Koshland et al. (Figure 2). According to this proposal, the mechanism occurs as a double

displacement involving two steps: a glycosylation and a deglycosylation step. In the first step, the enzyme is glycosylated by the concerted action of the carboxylates of two residues, either Asp or Glu, or both that are found on opposite sides of the enzyme active site and are normally close to each other (around 5.5 Å). One of these residues functions as a general acid in the first step of the mechanism where the glycosidic bond starts to break. The acid residue donates a proton to the dissociated sugar. During the same step, the second deprotonated carboxylate acts as a nucleophile, attacking the anomeric carbon at the oxocarbenium ion-like transition state. This step, referred to as the glycosylation step, leads to the formation of a covalently linked glycosyl-enzyme intermediate that has an anomeric configuration opposite to that of the starting material. The second step of this reaction, the deglycosylation step, involves the hydrolytic breakdown of the glycosyl-enzyme intermediate. The carboxylate that first functioned as an acid catalyst now acts as a base by abstracting a proton from the incoming nucleophile, usually a water molecule. Simultaneously, the water molecule attacks the carbohydrate-enzyme linkage in a reverse mode of the first step. At the end of the reaction, the enzymatic turnover is obtained and a hemi-acetal is formed with the same anomeric configuration as the starting material. Recent studies have shown that the transition states (TS1 and TS2) of both glycosylation and deglycosylation steps have a dissociative nature. Both reactions are favored by the distortion of the substrate during catalysis, but this effect is more evident in the first step of the reaction. The glycosylation process is also favored by the hydrogen bond between the nucleophilic carboxylate and the hydroxyl group of position 2 in the substrate. It behaves almost as an anchor that aligns the substrate in the active site and facilitates the glycosylation process.

A variation of the general mechanism for retaining enzymes has been demonstrated for the N-acetyl-β-hexosaminidases, belonging to families 18 and 20. Unlike the most retaining glycosidases, these enzymes lack a catalytic nucleophile, e.g. the water molecule. Instead, it is the acetamido substituent of the substrate that acts as an intramolecular catalytic nucleophile. As it is shown in Figure 3, the general acid/base residue protonates the oxygen of the scissile glycosidic bond. The other charged carboxylate residue stabilizes the positive charge developed on the nitrogen of the oxazolinium ion that is formed after the intramolecular attack of the N-acetamido oxygen to the anomeric carbon. To complete
the double displacement mechanism, in the second step, an incoming water molecule attacks the anomeric carbon, resulting in a product with retention of the initial configuration.\(^7\) In this reaction, several aromatic residues available in the active site have a key role to endorse the correct orientation of the nucleophilic carbonyl oxygen of the substrate and in this way promote and stabilize the formation of the oxazolinium ion.

Figure 3. Catalytic mechanism of retaining \(\beta\)-hexosaminidase from families GH18 and GH20.

2.2. Inverting GHs

In inverting GHs, there is an inversion of the anomeric configuration of the starting material. Here, the two crucial carboxylic residues act as general acid and base catalysts and these groups are \(\text{circa} 10.5\ \text{Å}\) apart from each other. It this specific case, this distance is larger than in retaining GHs because the substrate and the water molecule must be present simultaneously in the active site of the enzyme during the hydrolytic process.\(^{18,66,74-78}\) Figure 4 shows the proposed mechanism of action for inverting GHs, which occurs via a single-displacement type of mechanism. In this case, one of the carboxylate residues protonates the scissile glycosidic oxygen atom while the other coordinates the nucleophile (i.e. the water molecule) to assist its deprotonation and in this way complete the hydrolysis reaction.\(^7\)

Figure 4. Catalytic mechanism of inverting GHs.

In contrast with the retaining mechanism, this reaction is completed in a single step and it is supposed that it requires the formation of a single transition state structure. Moreover, it does not involve the formation of any covalent enzyme intermediate during the course of catalysis and induces the inversion of the anomeric configuration of the starting material.
2.3. Cofactor dependent GHs

There are other GHs whose catalytic mechanism is substantially different from the mechanisms described above. One of the most interesting ones requires the presence of an NADH cofactor. The retaining 6-phospho-α-GH enzymes from family 4 are among these enzymes (Figure 5) in which the cofactor is perfectly positioned to remove the hydride from carbon C3 of the substrate. Consequently, the acidity of the hydrogen atom that is attached to carbon C2 of the substrate increases and helps its abstraction by one of the tyrosine residues that is available in the active site and acts as a base. The hydroxyl group that is initially attached to carbon C3 of the substrate is subsequently oxidized to a ketone forming the 1,2-unsaturated reactive intermediate. Simultaneously, one of the carboxylate residues assists the cleavage of the sugar bound and the proton that is attached to carbon C2 of the substrate is abstracted by the base. This step is favoured by the presence of a metal ion in the active site of the enzyme that polarizes the carbonyl at carbon C3 and stabilizes the enolate species. The last step of this mechanism involves the nucleophilic attack of one water molecule to the double bond of the ketone. Simultaneously, the re-protonation of the enolate is catalysed by the close presence of the tyrosine residue, and the reduction of the ketone located at carbon C3 is accomplished by the NADH, favouring the enzymatic turnover.

Figure 5. Catalytic mechanism for NADH dependent GHs.

2.4. Transglycosylation activity of GHs

In addition to the hydrolytic ability, GHs can also be used under appropriate conditions for the reverse reaction, thus promoting the formation of glycosidic linkages. This type of
reactions are called transglycosylation\textsuperscript{62} and generally require high concentration of substrate. The proposed catalytic mechanism is depicted in Figure 6. Similarly to the previous described mechanisms, the first step leads to the departure of the aglycon group and the formation of the covalent intermediate. The second step of the reaction involves the attack of the carbohydrate-enzyme linkage by another sugar molecule, and the proton transfer from the sugar to the active site acid/base carboxylate.

![Figure 6. Transglycosylation reaction catalyzed by retaining GHs.](image)

Usually, the synthesis of glycosidic linkages in nature is carried out natively by glycosyltransferases (that use activated glycosides as the glycosyl donors). Typical glycoside donors are expensive nucleotide sugars such as ADP-glucose, UDP-glucose, and UDP-galactose. In contrast, the transglycosylation activity of GHs employs a considerably inexpensive substrate (such as simple sugars) as a glycoside donor molecule leading to large industrial interest in employing these enzymes for biotechnological synthesis. However, the yields for these transglycosylation reactions are typically low because the product itself is a substrate for the enzyme and undergoes hydrolysis. As their hydrolytic activities compete with this mechanistic pathway, it is necessary to displace the equilibrium of the glycosidic bond formation using excessive substrate concentration (thermodynamic control) or using good activated glycosyl donors, such as an aryl glycoside (kinetic control). Another disadvantage of the transglycosylation reactions catalysed by GHs is their limited efficiency for the glycosides synthesis in disaccharides or trisaccharides.\textsuperscript{81} This happens because these reactions require high degrees of chemo, regio and stereo-selectivity.\textsuperscript{82} The same is also true for oligosaccharides, but in this case the problems arise from their complex structure turning their chemical synthesis difficult to achieve, namely the production of glycosides with a mixture of various linkages (i.e., formation of 1-2, 1-3, 1-4 and 1-6 glycosidic bonds) and both anomers (\(\alpha\) and \(\beta\)).\textsuperscript{83} In this regard, the control of the stereospecificity and the regiospecificity of bond formation remains a challenging problem in the chemical synthesis of oligosaccharides.\textsuperscript{84,85} A solution for this unsolved problem would be very important, as industrially there is only interest in the oligosaccharide target.

In order to overcome most of the limitations of the transglycosylation reaction in glycosidases, many enzymes have been mutated in the region of the active site in order to enhance the rate of this reaction. A classical example of mutated glycosidases are the glycosynthases, in which the hydrolytic activity has been inactivated through the mutation of their catalytic nucleophile residues by small non-nucleophilic residues, such as alanine,
glycine or serine. These enzymes possess a high activity because they are able to accept an activated glycosyl donor group (generally glycosyl fluorides or nitropheryl glycosides) and catalyse transglycosylation reactions to an acceptor molecule.\(^8\)\(^1\)\(^8\)\(^5\) In opposition to the native GHs, these engineered enzymes produce carbohydrates with elevated molecular weight and with higher product yields.\(^8\)\(^1\)\(^ The first glycosynthase enzyme was reported in 1998 by Withers and colleagues\(^8\)\(^6\) but, currently many other glycosynthases have been developed that posses specific substrate specificity. Figure 7 shows the reaction mechanisms of several types of glycosynthases. As native glycosidases, the glycosynthases can also show retaining and inverting mechanism. In the inverting \(\alpha\)-glycosynthases, the donor group is transferred

\begin{figure}[h]
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\includegraphics[width=\textwidth]{glycosynthases.png}
\caption{Catalytic mechanism of glycosynthases: a) inverting \(\beta\)-glycosynthase, b) retaining \(\beta\)-glycosynthase, c) inverting \(\alpha\)-glycosynthase and d) thioglycoligase.}
\end{figure}
to the 4-nitrophenyl-α-glucoside acceptor group and the deglycosylation step proceeds similarly to what is observed with the retaining GHs (Figure 7a). The retaining glycosynthases act within the presence of one external nucleophile, such as sodium formate, and an activated donor group with the anomeric configuration of the native substrate (commonly 2-nitrophenyl- or 2,4-dinitrophenyl-β-glucoside) (Figure 7b). Therefore, the nucleophile mimics the catalytic active-site carboxylate of the enzyme and builds the formyl-glycoside intermediate. Subsequently, the donor carbohydrate is transferred to an acceptor sugar, promoting the polysaccharide synthesis.85,87-90

Some retaining glycosynthases can also have inverting mechanisms. This occurs when the donor sugar has a glucosyl fluoride in an opposite anomeric configuration relatively to the native substrate, thus mimicking the intermediate of the reaction (Figures 7a and 7c).86

Another type of glycosynthases are the thioglycoligase engineering enzymes, in which the mutated residue is the acid/base carboxylate instead of the nucleophile residue as in the previously described glycosynthases (Figure 7d). In these cases, a good leaving group such as dinitrophenyl, is placed in the substrate, which allows the formation of the glycosyl-enzyme intermediates that favors the catalytic process.91

3. Structural aspects that influence the GHs catalytic mechanism

The structural studies addressed at GHs have also provided important clues about how these enzymes enhance the catalytic process. As mentioned before, the distortion of the substrate along the full catalytic process is one of these mechanisms and this effect is found in many studies.92-99 The available data reveals that GHs are able to selectively bind and stabilize high energy substrate conformations before the hydrolysis takes place. Such distortion of the substrate favours, in the Michaelis complex, the attack of the catalytic acid/base carboxylates to the glycosidic oxygen of the substrate. At the same time, it helps to guide the leaving group in a pseudoaxial position in relation to the substrate, facilitating the nucleophilic attack on carbon C1 and the subsequent cleavage of the glycosidic bond. It has been proposed also that the conformation changes of the ring along the catalytic process might determine the efficiency of the polysaccharides degradation. Taking into account these results and the available X-ray structures of several GHs that contain the substrates with different conformational distortions, Stoddart100-102 proposed a diagram to classify the conformation of a α-glucopyranose molecule ring along the reaction pathway (Figure 8). In this diagram C, B, S corresponds to the chair, boat, and skew conformations, respectively. This diagram includes the most energetically stable 4C1 chair, six boat-type and six skew-type conformations, as well as several transient structures (e.g. half-chair and envelope conformations) between the transition of 4C1 chair to the boat/skew conformations.

The itinerary map of Stoddart gives therefore all the possible conformational pathways that a hypothetical substrate may follow as it moves from one conformation to another94. However, no energetically information can be extracted regarding the relative stability of different conformations, nor can it be assumed that all conformations on this map correspond to stationary points in the free energy landscape with respect to ring distortion.
Nevertheless, over the years, this diagram has been actively used as an “itinerary map” to design new enzyme inhibitors for therapeutic activities. In this regard, the conformational itinerary pathway of several GHs families has been studied, such as GH29 enzymes and α-xylosidases from the GH31 family that catalyze the hydrolysis using the $4C_1 \leftrightarrow 3H_4 \leftrightarrow 2S_1$ glycosylation itinerary \(^{93,103}\); inverting endoglucanases from the GH8 family that use the $\beta-2S_0/2,5B \leftrightarrow 2,5B \leftrightarrow \alpha-5S_1$ glycosylation itinerary of the glycon ring \(^{104}\); the glycosylation reaction of golgi α-mannosidase II from the GH38 family following an $0S_2/B_2,5 \leftrightarrow B_2,5 \leftrightarrow 0S_1$ itinerary \(^{105}\); the catalytic itinerary of 1,3-1,4-β-glucanase from the GH16 family 16 pursue the $1,4B/1S_3 \leftrightarrow 4E/4H_5 \leftrightarrow 4C_1$ \(^{95,106}\) itinerary, and the inverting α-mannosidases from the GH47 family that follow the $2S_1 \leftrightarrow 3H_4 \leftrightarrow 4C_1$ glycosylation itinerary.\(^{107}\)

Many GHs also contain cations in the region of the active site. The presence of these species in the structure of GHs appears to be more common than it was initially imagined, and are believed to be very important for the stabilization of the transition states during catalysis. For instance, the Golgi α-mannosidase II from the GH38 family has a zinc ion in its 3D structure. Studies on the catalytic mechanism of this enzyme revealed that the Zn\(^{2+}\) ion is fundamental to coordinate the hydroxyl groups attached to carbons C2 and C3 of the mannosyl group, which stabilize the transition states, and thus reduces the overall activation energy required for the reaction. Furthermore, QM/MM metadynamics simulations also demonstrate that the zinc ion helps to lengthen the C2 hydroxyl bond when the substrate acquires the oxocarbenium character, facilitating the electron reduction of this species.\(^{105}\) A similar role has been proposed for the calcium ion present in the structure of the endoplasmic reticulum α-mannosidase I from the GH47 family. The crystallographic structure shows that the cation coordinates with the hydroxyl groups that are attached to
carbons C2 and C3 of 1-deoxymannojirimycin or kifunensin inhibitors. A magnesium ion is also found close to the region of the active site of β-galactosidase from *Escherichia coli*. In these case, theoretical calculations have clearly demonstrated that the presence of the cation has a key role in lowering down the activation barrier by 14.9 kcal/mol, emphasizing its importance during the catalytic process.

4. Conclusions

GHs are impressive nano-molecular machines that are present in almost all living organisms (exceptions are some Archaeans and a few unicellular parasitic eukaryotes). These enzymes catalyze the hydrolysis of the glycosidic linkage in a myriad of biological reactions and under specific conditions can also catalyse the reverse reaction promoting the formation of glycoside linkages.

The interest of GHs has started long ago but the catalytic power behind glycosidases is only now being established. Structural analyses of various enzyme complexes representing stable intermediates along the reaction coordinate together with detailed mechanistic and spectroscopic studies on wild type and mutant enzymes, have revealed that the source for their catalytic power is based on nucleophilic and general acid/base catalysis. These enzymes develop finely tuned active sites that contain two carboxylates residues (Asp and Glu) carefully aligned and positioned on opposite sides of the enzyme active site that embrace the substrate upon substrate binding. The active site also provides an extensive network of hydrogen bonds that endorse a conformational distortion of the substrate. This induces the substrate to adopt a higher energetic conformation before the hydrolysis takes place, and such configuration is maintained during the full catalytic process. This effect is very important for the stabilization of the transition-state structure and therefore to lower the activation barrier of the full process. Some GHs also possess positive ions (Zn²⁺, Mn²⁺, and Mg²⁺) into their structure and these cations have been found also to be essential for the stabilization of the transition states during catalysis. The two most commonly employed mechanisms used to hydrolyze the glycoside linkage of substrates by glycosidases involve the retention or the inversion mechanisms. These mechanisms are conserved within the majority of GHs families. The only exception are the glycosidases from family 4 and 109, in which the hydrolytic process occurs via an elimination type of mechanism, and requires the presence of the NADH cofactor.

The structural and the mechanistic studies addressed to glycosidases provided and continue to provide important clues about the catalytic power of these enzymes. This knowledge is very important as it offers new ways to improve, modify or even inhibit the activity of GHs. These developments are particularly important for the biotechnology industries that have been increasing the commercial uses of glycosidases in several areas. Indeed, specific glycosidases are increasingly used for food processing, for bio-bleaching in the pulp and paper industry, as well as for biomass degradation with the potential to convert solid biomass into liquid fuels.
In the last two decades, it has also been watched an increasing interest of glycosidases for therapeutic uses. Glycosidases are involved in the biosynthesis of the oligosaccharide chains and quality control mechanisms in the endoplasmic reticulum of the N-linked glycoproteins. Inhibition of these glycosidases can have profound effects on quality control, maturation, transport, and secretion of glycoproteins and can alter cell–cell or cell–virus recognition processes. This principle is the basis for the potential use of glycosidase inhibitors in viral infection, diabetes and genetic disorders. Most of these drugs are glycosidase inhibitors that can bind and block the active sites of these enzymes. Some successful examples are the α-amylase inhibitor Acarbose and Miglitol that were approved by the FDA in 1990 and 1996, respectively, and are used to inhibit some of the intestinal glycosidases and pancreatic α-amylase in order to regulate the absorption of carbohydrates. These inhibitors are currently used therapeutically in the oral treatment of the non-insulin-dependent diabetes mellitus (type II diabetes). Other glycosidases inhibitors are used as anti-viral agents. Here the inhibitors were developed to inhibit the formation of glycoproteins of the viral envelopes, which are essential for virion assembly and secretion and/or infectivity. A successful example was the development of Zanamivir (Relenza) and Oseltamivir (Tamiglu), approved by FDA in 1999, that are used in the treatment and prophylaxis of influenza caused by influenza A virus and influenza B virus. These compounds efficiently inhibit a glycosidases called neuraminidase. Glycosidases are also used in the therapy of human genetic disorders. The glycosphingolipid storage diseases (GSD, also glycogenosis and dextrinosis) are a rare hereditary disorders that are severe in nature and frequently fatal. These diseases result from defects in the processing of glycogen synthesis or breakdown within muscles, liver, and other cell types. An example of such disorders is the Fabry disease that is caused by the deficiency of the essential enzyme α-glycosidase A, resulting in renal failure along with premature myocardial infarction and strokes. The only successful treatment is, to date, the enzyme replacement therapy. Fabrazyme was approved by FDA in 2003 and is intended to replace the missing enzyme in patients with this progressive disease.

Taking into account that almost two-thirds of all carbon that exist in the biosphere is carbohydrate, we believe that the current applications of GHs are only a small group of the many very important applications that these enzymes may find in the future. It is therefore expected that a wide variety of relevant and new applications will arise in the near future involving glycosidases. To stimulate these developments, the continuous study regarding glycosidases will be very important as they will provided crucial knowledge to turn their use more efficient and effective.

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