

Abstract

Glycosidases catalyse transformations leading to the attachment of carbohydrate molecules to aglycons. Hence, a detailed description of glycosidases is made in this chapter which includes their classification, nature, source, structural features, mechanism of glycosylation and advantages of such reactions. Also mentioned are examples of glycosylation reactions involving a wide variety of aglycons with different carbohydrate molecules in the form of a table.

2.1 Introduction

In order to carry out an enzymatic transformation reaction, one requires a profound knowledge on enzymes themselves. Although several enzymes have been employed in such reactions, this book will deal mainly with two most well-known hydrolytic enzymes, glycosidases and lipases. A detailed description on glycosidases is outlined in this chapter.

Among the enzymes dealing with carbohydrates, glycosidases and transglycosidases play an important role in the synthesis of glycosides. They belong to the group of carbohydrate-processing enzymes, widely employed in the regio- and stereoselective glycosylation reactions. Glycosidases are carbohydrases – enzymes that catalyse the hydrolysis of glycosidic bonds to liberate monosaccharides and oligosaccharides of lower molecular weight than the native simple as well complex carbohydrate substrates. These

enzymes are very widely distributed in nature and found in all organisms. These large and important groups of enzymes, now known as amylase, were investigated long back by Payen and Persoz (1833), who were probably the first to recognise this enzyme in 1833 as ‘diastase’ (1833). Subsequently, a detailed study on glycosidases was carried out by many eminent chemists and biochemists including Fischer (1894).

2.2 Amylolytic Enzymes

Starch-degrading enzymes have been broadly classified into two groups – endo-acting enzymes or endohydralases and exo-acting enzymes or exohydralases (Berfoldo and Anthranikian 2001). α -Amylase (α -1,4-glucan-4-glucanohydralase; EC 3.2.1.1) is an endo-acting enzyme which hydrolyses linkages in the starch polymer chain randomly, leading to the generation of linear and

branched oligosaccharides. Most starch-hydrolyzing enzymes belong to the α -amylase family containing a characteristic catalytic $(\beta/\alpha)_8$ barrel domain. Exo-acting starch hydrolases such as β -amylase, glucoamylase, α -glucosidase and isoamylase attack the substrate from the nonreducing end, producing oligosaccharides. β -Amylase (EC 3.2.1.2), also referred to as α -1,4-D-glucan maltohydrolase or saccharogen amylase, hydrolyses α -1,4-glucosidic linkages of the starch chain to liberate successive maltose units from the nonreducing end, thereby producing β -maltose units by an inversion of configuration. α -Glucosidase (EC 3.2.1.20) attacks α -1,4 linkages of oligosaccharides and liberates glucose by retaining α -anomeric configuration.

2.3 Glucoamylase

Glucoamylase (E.C 3.2.1.3) is a fungal enzyme which goes under the names amyloglucosidase, 1,4- α -D-glucan hydrolase and γ -amylase. Enzyme code assigned for this enzyme by the Enzyme Commission (IUBMB 1992) is EC 3.2.1.3 where number 3 denotes hydrolases, referring to catalytic hydrolytic cleavage of large molecules with the addition of water; number 2 indicates glycosidic bond-cleaving glucosidases, and number 1 refers to hydrolysis of O-glycosyl compounds. There are several enzymes under the group 3.2.1, of which glucoamylase is number 3 which forms the fourth number in the nomenclature. Glucoamylase refers to hydrolysis of terminal α -1,4-linked-D-glucose residues successively from nonreducing ends of the carbohydrate chains from starch and malto-oligosaccharides, releasing D-glucose with inversion of configuration to β -D-glucose (Fogarty 1983).

When the next bond sequence is α -1,4, most forms of the enzyme can hydrolyse α -1,6-D-glucosidic bonds also. However, in vitro, this enzyme hydrolyses α -1,6- and α -1,3-D-glucosidic bonds also in other polysaccharides with high molecular weights. Since this enzyme is capable of completely hydrolyzing starch under long incubation periods, it is also called the saccharifying enzyme. Glucoamylases have the capacity to degrade large

oligosaccharides up to about 90% α -1,6 linkages depending on the size of the substrate and the position of the α -1,6 linkages. Reverse reactions involving synthesis of saccharides and glycosides from D-glucose occur with a very high glucoamylase concentration for prolonged incubation periods and high concentrations of substrates.

2.4 Sources of Glucoamylases

The main source of glucoamylases is fungi although they are derived from a wide variety of plants, animals and microorganisms. Commercial enzymes originate from strains of either *Aspergillus niger* or *Rhizopus* sp. where they are used for the conversion of malto-oligosaccharides into glucose (Fogarty 1983). Since the discovery of two forms of glucoamylase from black koji mould in the 1950s, many reports have appeared on the multiplicity of glucoamylases, envisaged to be the result of several mechanisms, namely, mRNA modifications, limited proteolysis, variation in carbohydrate content or presence of several structural genes (Pretorius et al. 1991).

Fungal glucoamylases are usually one to five forms of glycoproteins. *Aspergillus niger* is being used widely in the commercial production of an extracellular glucoamylase. Two forms of glucoamylase – AG-I (glucoamylase I, 99 kDa) and AG-II (glucoamylase II (112 kDa) – isolated from *A. niger* differed in their carbohydrate content, pH, temperature stabilities and activity (Williamson et al. 1992; Stoffer et al. 1993).

Glucoamylase from *Aspergillus terreus* strains was examined for the production of D-glucose and corn syrups (Ghosh et al. 1990; Ali and Hossain 1991). A glucoamylase from *Rhizopus* sp. released glucose from starch with 100% efficiency (Yu and Hang 1991). Takahashi et al. (1985) isolated three forms of glucoamylase from *Rhizopus* sp., GA-I (74 kDa), GA-II (58.6 kDa) and GA-III (61.4 kDa). Glucoamylases from other mould strains are *Humicola lanuginosa* (Taylor et al. 1978), *Thermomyces lanuginosa* (Haasum et al. 1991), *Myrothecium* sp. M1 (Malek and Hossain 1994) and a phytopathogenic fungus *Colletotrichum gloeosporioides* (Krause et al. 1991).

There are several reports on the production of yeast glucoamylases (Saha and Zeikus 1989; Pretorius et al. 1991). Glucoamylase has been identified in *Saccharomyces cerevisiae* (Pugh et al. 1989), *Saccharomyces cerevisiae* var. *diastaticus* (Kleinman et al. 1988; Pretorius et al. 1991), *Saccharomycopsis fibuligera* (Itoh et al. 1989), *Schwanniomyces castellii* (Sills et al. 1984), *Schwanniomyces occidentalis* (Gellissen et al. 1991), *Pichia burtonii* and *Talaromyces* sp.

Bacterial glucoamylases have also been identified from aerobic strains such as *B. stearrowthermophilus* (Srivastava 1984), *Flavobacterium* sp. (Bender 1981), *Halobacterium sodomense* (Chaga et al. 1993) and *Arthrobacter globiformis* I42 (Okada and Unno 1989). Anaerobic strains include *Clostridium thermohydrosulfuricum* (Hyun and Zeikus 1985), *Clostridium* sp. G0005 (Ohinishi et al. 1991), *Clostridium acetobutylicum* (Chojacki and Blaschek 1986; Soni et al. 1992), *Clostridium thermosaccharolyticum* (Specka et al. 1991) and the microaerophile, *Lactobacillus amylovorus* (James and Lee 1995).

2.5 Sources of Other Glycosidases

Among the thermostable glycosidases used in the synthesis of glycosides, the most remarkable one is the β -glucosidase from the hyperthermophilic archeon *Pyrococcus furiosus* (Kengen et al. 1993) which is relatively easy to grow, and the enzyme is stable for 85 h at 100 °C. The enzyme has been cloned and over-expressed in *Escherichia coli* (Voorhorst et al. 1995). β -Galactosidase from *Aspergillus oryzae* was efficient towards alkylation (Stevenson et al. 1993). β -Galactosidase from *Streptococcus thermophilus* (Stevenson and Furneaux 1996) was employed for the synthesis of ethyl glycoside. β -Galactosidase from *Bacillus circulans* was also exploited by number of workers for synthetic purposes (Kojima et al. 1996). Enzymatic synthesis of butylglycoside via a transglycosylation reaction of lactose was carried out using β -galactosidase from *A. oryzae* (Ismail et al. 1999a). With primary as well as secondary alcohols, β -xylosidase from *A. niger* is an efficient glycosyl transfer catalyst that gave high (> 80%)

yields of alkyl xylosides (Shinoyama et al. 1988) from methanol up to butanol. Almond glucosidase has been widely employed for the synthesis of alkyl and phenolic glycosides (Ljunger et al. 1994; Vic and Crout 1995; Vic et al. 1995; Ducret et al. 2002).

2.6 Structural Features of Glucoamylase

The structure of different glucoamylases showed a common subsite arrangement with seven in total and the catalytic site was located between subsite 1 and 2 (Hiromi et al. 1973; Ohinishi 1990; Fagerstrom 1991; Ermer et al. 1993). Subsite 2 has the highest affinity for oligomeric substrates and glucose, followed by decreasing affinity towards subsites 3–7 (Fagerstrom 1991). The glucoamylase G1 of *A. niger* consists of three parts: (1) Ala-1-Thr-440, containing the catalytic site; (2) Ser-441-Thr-551, a highly O-glycosylated linker segment; and (3) Pro-512-Arg-616, a C-terminal domain responsible for substrate binding (Stoffer et al. 1995; Svensson et al. 1983). Functionally important carboxyl groups in glucoamylase G2 from *A. niger* were identified to be Asp176, Glu179 and Glu180 in the catalytic site (Svensson et al. 1990). Tryptophan residues have been proposed to be essential for enzymatic activity (Rao et al. 1981) in *A. niger* glucoamylase and essentially tryptophan120 is reported to be responsible for binding of substrate and maintaining the structural integrity necessary for catalysis (Clarks and Svensson 1984).

Aspergillus awamori GA-I has also three catalytic domains (Svensson et al. 1983) like *A. niger*, a catalytic domain (residues 1–440), an O-glycosylated domain (residues 441–512) and a starch-binding domain (residues 513–616). Aleshin et al. (1994) produced a structural model for the catalytic domain of glucoamylase from *A. awamori* from a 2.2-Å resolution crystal structure of a proteolized form of GA-I *A. awamori* var *X100*, which contained the complete catalytic domain plus GA-II domain the N-terminal half of the O-glycosylated domain (residue 1–471). Amino acid sequence of three glucoamylases from

Rhizopus, *Aspergillus* and *Saccharomyces* were compared (Tanaka et al. 1986), of which the glucoamylases from *Rhizopus* and *Aspergillus* were highly homologous in both the nucleotide sequence and the amino acid sequence suggesting that these two glucoamylases were the most closely related among the three. The catalytic site in glucoamylase is believed to consist of two carboxyl groups (Hiromi et al. 1966a, b), where one acts as a general acid, protonating the glucosidic oxygen, while the other in the ionised carboxylate form stabilises the substrate intermediary oxonium ion (Braun et al. 1977; Matsumura et al. 1984; Post and Karplus 1986; Rantwijk et al. 1999).

Itoh et al. (1989) reported that in *S. fibuligera* glucoamylase, Ala-81, Asp-89, Trp-94, Arg-96, Arg-97 and Trp-166 were required for wild-type levels of activity, and Ala-81 and Asp-89 were not essential for catalytic activity which however played a role in thermal stability.

Complexes of glucoamylase from *A. awamori* with acarbose and D-gluco-dihydroacarbose indicate hydrogen bonds between sugar OH groups and Arg-54, Asp-55, Leu-177, Try-178, Glu-180 and Arg-305 of subsites 1 and 2 (Aleshin et al. 1994; Stoffer et al. 1995). Glu-179 (Sierks et al. 1990) and Glu-400 are positioned geometrically for general acid and base catalysis, ideal for the glucoside bond cleavage and assistance in the nucleophilic attack of water at the anomeric centre of the carbohydrate (Harris et al. 1993; Frandsen et al. 1994). Both the active sites of *A. niger* and *Rhizopus oryzae* glucoamylases are very much identical (Stoffer et al. 1995). In the active site of *R. oryzae*, the amino acid residues Arg-191, Asp-192, Leu-312, Trp-313, Glu-314, Glu-315 and Arg-443 are responsible for substrate binding through hydrogen bonds, whereas Glu-314 and Glu-544 are for glucosidic bond cleavage (Ashikari et al. 1986; Sierks et al. 1990).

2.7 Structural Features of β -Glucosidase

Sweet almond β -glucosidase has been known to hydrolyse glycosides resulting in the net retention of anomeric configuration (Eveleigh and

Perlin 1969). It has followed the standard mechanism of such retaining glycosidases (McCarter and Withers 1994; Sinnot 1990). Assignment of sweet almond β -glucosidase as a family 1 glycosidase and identification of its active site nucleophilic residues sequence Ile-Thr-Glu-Asn-Gly were done by He and Withers (1997).

The primary structures of maize and sorghum β -glucosidases possess highly conserved peptide motifs TENEP and ITENG, which contain the two glutamic acids (Glu-191 and Glu-406) involved in the general acid/base catalysis and the respective family 1 β -glucosidases nucleophiles (San-Aparicio et al. 1998). A part slot-like active site (Davies and Henrissat 1995) was formed by these residues necessary for the substrate hydrolysis (Withers et al. 1990).

In the glycosylation step, the nucleophile Glu-406 attacks the anomeric carbon (C-1) of the substrate and forms a covalent glycosyl-enzyme intermediate with concomitant release of the aglycon after protonation of the glucosidic oxygen by the acid catalyst Glu-191 (Withers et al. 1990). In the next deglycosylation step, Glu-191 acts as a base, and a water molecule functions as the nucleophile and attacks the covalent glycosyl-enzyme, releasing the glucose and regenerating the nucleophilic Glu-406. In maize β -glucosidase isozyme Glu-1, these two catalytic glutamic acids are positioned within the active site at expected distances of ~ 5.5 Å for this mechanism (Czjzek et al. 2001). Verdoucq et al. (2003) from co-crystals of enzyme substrate and enzyme aglycon complexes of maize β -glucosidase isozyme Glu1 (ZmGlu1) have shown that five amino acid residues – Phe-198, Phe-205, Try-378, Phe-466 and Ala-467 – are located in the aglycon-binding site of ZmGlu1 which form the basis of aglycon recognition and binding and hence the substrate specificity. Kaper et al. (2000) have studied the substrate specificity of a family 1 glycosyl hydrolase – the β -glucosidase (CelB) from the hyperthermophilic archaean *Pyrococcus furiosus*, at a molecular level exhibiting a homotetramer configuration, with subunits having a typical $(\beta\alpha)_8$ -barrel fold. Comparison of the 3D model of the *Pyrococcus furiosus* β -glucosidase and the 6-phospho- β -glucosidase (LacG) from

the mesophilic bacterium *Lactococcus lactis* (Kaper et al. 2000) showed that the positions of the active site residues in LacG and CelB are very well conserved, and the conserved residues involved in substrate binding are Asn-17, Arg-77, His-150, Asn-206, Tyr-307 and Trp-410. The average distance between the oxygen atoms of these glutamate carboxylic acids is 4.3 Å (± 1 Å) in CelB, which is very much in the range of the general observed distance in retaining glycosyl hydrolases (McCarter and Withers 1994).

Investigation by Hays et al. (1998) of the catalytic mechanism, substrate specificity and transglycosylation acceptor specificity of guinea pig liver cytosolic β -glucosidase (CBG) indicated that CBG employed a two-step catalytic mechanism with the formation of a covalent enzyme-sugar intermediate and that CBG transferred sugar residues to primary hydroxyls and equatorial but not axial C-4 hydroxyls of aldopyranosyl sugars (Hays et al. 1998). Also the specificity of CBG for transglycosylation reactions was different from its specificity for hydrolytic reactions (Hays et al. 1998) and that CBG possessed a single active site nucleophile, specifically the glutamate residue in the sequence TITENG.

2.8 Glycosylation

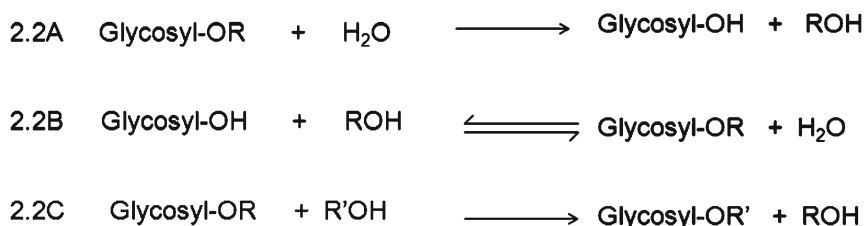
Hydrolysis is the natural reaction for glucosidases and glucoamylases, whereas glycosylation is a forced, reversed reaction. Glycosides are asymmetric mixed acetals formed by the reaction of the anomeric carbon atom of the intermolecular hemiacetal or pyranose/furanoses form of the aldohexoses or aldoketoses with a hydroxyl group furnished by an alcohol (Lehinger 1975; Ernst et al. 2000). The bond formed is called glycosidic bond, and the reaction is called glycosylation. Because of multiple hydroxyl groups of similar reactivity, controlled glycosylation remains a challenge to organic chemists. Classical chemical approaches inevitably require quite a number of protection, activation, coupling and deprotection steps (Igarashi 1977; Konstantinovic et al. 2001). In contrast, enzymes (glycosidases and transglycosidases) offer one-step synthesis under mild

conditions in a regio- and stereoselective manner (Vic and Thomas 1992). Enzyme-catalysed glycoside and oligosaccharide synthesis involves two types of reaction – a reverse hydrolytic glycosidase and a glycosyl-transferase-catalysed glycoside bond formation. A sugar donor and acceptor are incubated with the appropriate glycosidase or glycosyl-transferase that catalyses the efficient and selective transfer of the glycosyl residue to the acceptor. Glycosyl-transferases are often difficult to obtain (Auge et al. 1990), while, in contrast, the glycosidase approach uses simpler glycosyl donors, the free monosaccharide itself. This method has the advantage of using relatively simple glycosyl donors and readily available commercial enzymes at the expense of the absence of region selectivity in some instances (Trincon et al. 2003).

There are three types of reactions catalysed by glycosidases such as hydrolysis, reverse hydrolysis and transglycosylation (Scheme 2.1). In aqueous media, when there is large excess of water, glycoside or oligosaccharide or polysaccharide, hydrolysis is the dominant reaction (Scheme 2.1A). Other two reactions, namely, reverse hydrolysis and transglycosylation, lead to synthesis of glycosides, and the difference depends on the nature of the glycosyl donor.

The reverse hydrolytic approach is an equilibrium-controlled synthesis where the equilibrium is shifted towards synthesis (Panintrarux et al. 1995; Vic et al. 1997; Rantwijk et al. 1999) of a glycoside from a carbohydrate and an alcohol (Scheme 2.1B). This can be achieved by reducing the water activity, increasing the substrate concentrations and removing, if possible, the products of reaction (Vic and Crout 1995). This is a widely employed method for the enzymatic synthesis of alkyl glycosides and phenolic glycosides in an organic co-solvent (Vic and Crout 1995; Vic et al. 1997; Ducret et al. 2002).

The transglycosylation method is a kinetically controlled synthesis where the enzyme catalyses the transfer of a glycosyl residue from a glycosyl donor to the glycosyl acceptor (Scheme 2.1C). The reaction yield depends on the relative rate of product synthesis to that of hydrolysis. An efficient acceptor used in a high concentration



(Glycosyl = glycopyranosyl moiety, ROH & R'OH = alcohol)

Scheme 2.1 Reactions catalysed by glycosidases

should favour the synthesis (Ismail et al. 1999b; Rantwijk et al. 1999; Vulfson et al. 1990) although this may not be true with all the acceptors.

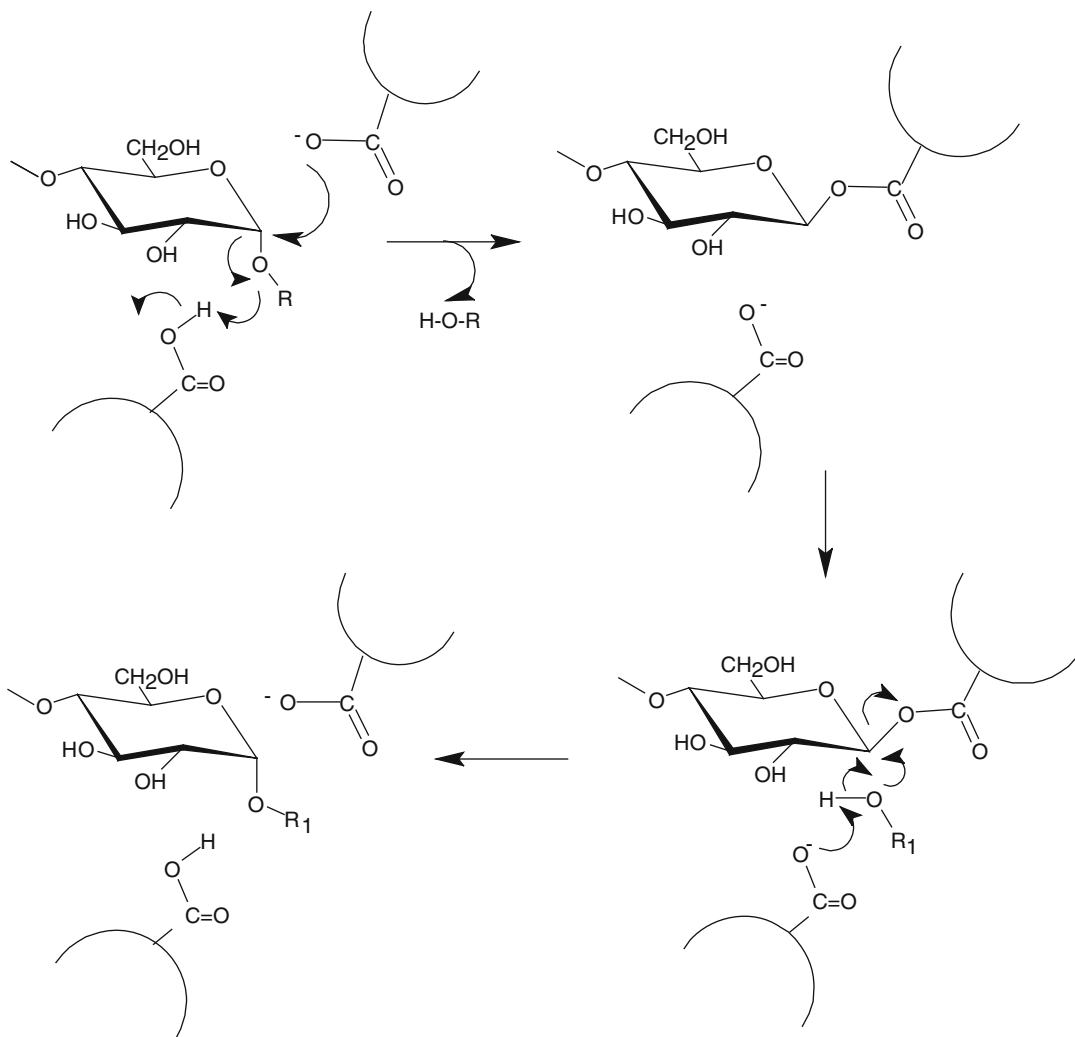
2.9 Mechanism of Glycosylation

In general, every hydrolysis of a glycosidic linkage by glycosidase is a reaction in which the product retains ($\alpha \rightarrow \alpha$ or $\beta \rightarrow \beta$) or inverts ($\alpha \rightarrow \beta$ or $\beta \rightarrow \alpha$) the anomeric configuration of the substrate (Chiba 1997). In the normal hydrolytic reaction, the leaving group is an (oligo)saccharide and the nucleophile (glycosyl acceptor) is water (Scheme 2.1A). However, an alcohol or a monosaccharide can also act as a glycosyl acceptor (glycosylation). In the reversed hydrolysis, the condensation of a monosaccharide and an alcohol in which water is the leaving group (Scheme 2.1B) was first reported in 1913 (Rantwijk et al. 1999). A recent review by Zechel and Withers (2001) focuses on the recent developments in the understanding of nucleophilic and general acid–base catalysis in glycosidase-catalysed reactions. Various models have been proposed for the catalytic reaction mechanisms of carbohydrate hydrolase in the transition state, but an unequivocal model remains to be established. Two significant models, such as nucleophilic displacement mechanism (Scheme 2.2) and an oxo-carbenium ion intermediate mechanism (Scheme 2.3), were suggested for the hydrolytic reaction where glycosyl acceptor is water (Chiba 1997).

The double displacement mechanism was found to be applicable to the enzymes, which

retain the anomeric configuration of the substrate. The two catalytic ionisable groups, a carboxyl, $-\text{COOH}$, and a carboxylate, $-\text{COO}^-$, cleave the glucosidic linkage cooperatively by direct electrophilic and nucleophilic attacks against the glycosyl oxygen and anomeric carbon atoms, respectively, resulting in a covalent glucosyl–enzyme complex by a single displacement. Subsequently glucosyl–acetal bond is attacked with the hydroxyl group of the water (alcohol hydroxyl group in glycosylation) by retaining the anomeric configuration of the product by the double displacement. The double displacement mechanism is adequate for explaining the reaction, where the anomeric configuration of the substrates is retained (Chiba 1997).

In the oxo-carbenium intermediate mechanism, the two catalytic groups of the carboxyl and carboxylate ion participate cooperatively in the departure of the leaving group by a proton transfer to the anomeric oxygen atom (Scheme 2.3). An enzyme-bound oxonium ion intermediate has been detected by NMR (Withers and Street 1988). The second carboxylate, which is deprotonated in the resting state, stabilises the oxonium ion intermediate. In the next step, a nucleophile adds to the same face of the glycosyl–enzyme intermediate from which the leaving group was expelled, resulting in the net retention of the anomeric configuration at the anomeric centre. The addition of the nucleophile is assisted by the first carboxylate which in this step reverts to carboxylic acid. The oxo-carbenium intermediate mechanism has been applied to interpret the catalytic mechanism of many carbohydrate-degrading enzymes. This



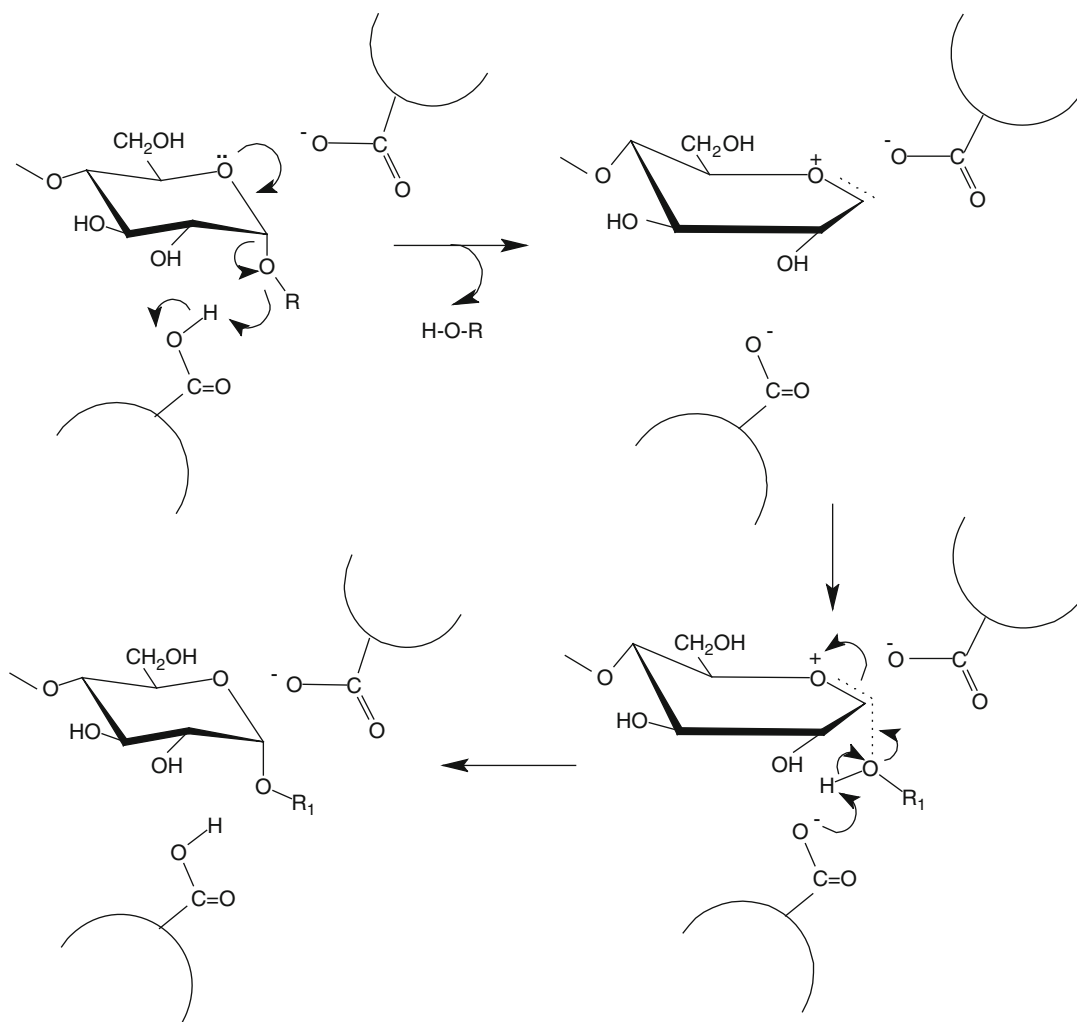
Scheme 2.2 Nucleophilic double displacement mechanism (Chiba 1997)

mechanism is applicable to both ‘retaining’ and ‘inverting enzymes’ (Chiba 1995). Mutagenesis and X-ray structural studies have confirmed that the mechanism of retaining glycosidases is similar (Sinnot 1990; Jacobson et al. 1994, 1995).

2.10 Glycosylation Reactions

Biological activities of a naturally occurring glycoside (Robyt 1998; Schmid et al. 2001; Akao et al. 2002) are primarily due to an aglycon moiety of that molecule. It is generally accepted that

glycosides are more water-soluble than most of the respective aglycons. Attaching a glycosidic moiety into the molecule increases its hydrophilicity and thereby influences physicochemical and pharmacokinetic properties of the respective compound like circulation, elimination and concentrations in the body fluids (Kren 2001). Glycosides with unsaturated alkyl chains like terpenes are claimed to possess antifungal and antimicrobial activity (Tapavicza et al. 2000; Zhou 2000) although it is unclear why the activity of these aglycons is improved by glycosylation. Chemical preparation of glycosides cannot meet



Scheme 2.3 Oxo-carbenium ion intermediate mechanism (Chiba 1997)

EC food regulations, and therefore, chemical preparation of glycosides is not applicable in the food industry.

Many glycosides are used in broad range of applications as surfactants (Busch et al. 1994), as food colourants and flavouring agents (Sakata et al. 1998), sweeteners (Shibata et al. 1991), antioxidants, anti-inflammatory (Gomes et al. 2002), antitumor (Kaljuzhin and Shkalev 2000), antibiotics (Ikeda and Umezawa 1999), antifungal (Tapavicza et al. 2000), antimicrobial (Zhou 2000) and cardiac-related drugs (Ooi et al. 1985). Glycosylation renders lipophilic compounds

more water-soluble and thereby increases bio-availability of biologically active compounds besides imparting stability to the aglycon (Kren and Martinkova 2001). Alkyl glycosides are mainly used as nonionic surfactants in food, pharmaceuticals, chemical, cosmetic and detergent industries. These types of nonionic surfactants exhibit several interesting properties in detergency, foaming, wetting, emulsification and antimicrobial effect (Matsumura et al. 1990; Balzar 1991). Alkyl glycosides are non-toxic, non-skin-irritating and biodegradable (Matsumura et al. 1990; Busch et al. 1994; Madsen et al.

1996). Further alkyl glycosides are used as raw materials for sugar fatty acid ester synthesis (Mutua and Akoh 1993).

2.11 Advantages of Enzymatic Glycosylation over Chemical Methods

There are many advantages of using glycosidases (Vijayakumar 2007; Sivakumar 2009):

1. Exploitation of regio- and stereospecificity and selectivity
2. Milder reaction conditions
3. Non-generation of by-products associated with the use of several chemical procedures
4. Improved product yield and better product quality
5. Use of nonpolar solvents which impart stability to glycosidases, renders insolubility of the enzyme, solubility of alcohols and products in organic solvents and easy product workout procedures
6. No protection activation and deprotection required
7. Less environmental pollution

The use of organic solvent in enzyme catalysis has attracted much attention due to several desir-

able factors such as solubilities of the organic compounds, shifting equilibrium towards the synthesis, increasing the enzyme stability and recovery of the enzyme (Rubio et al. 1991; Mohri et al. 2003). Poor solubility of the carbohydrate substrate in the organic phase is a limiting factor especially when hydrophobic alcohol (glycosyl acceptor) itself is used as a substrate and in some cases as a solvent media (Laroute and Willemot 1992; Vic and Crout 1995; Crout and Vic 1998). There are reports where glycosylations were carried out either in biphasic systems of a water-immiscible alcohol and water (that maintains sugar substrate and enzyme) or water and water-miscible monophasic system (Monsan et al. 1996). The process of glycosylation can be effected under nonaqueous, solvent-free, high-substrate, high-temperature and moderate to high water activity conditions to achieve good yield of glycosides (Nilsson 1987; Roitsch and Lehle 1989; Gyax et al. 1991; Laroute and Willemot 1992; Vic and Thomas 1992; Shin et al. 2000).

Table 2.1 lists some of the important surfactants, phenolic, flavonoid, terpinyl, sweetener and medicinal glycosides, which have been prepared by the use of glycosidases, glucoamylases and glycosyl-transferases.

Table 2.1 Glycosides from enzymatic glycosylations

Name of the compound	Source of enzyme	Applications	References
<i>A. Surfactant glycosides</i>			
(1) β -D-Glycopyranosides of n-heptanol, n-octanol, 2-phenyl hexanol, 3-phenyl propanol, 4-phenyl butanol, 5-phenyl petanol, 6-phenyl hexanol, 2-pyridine methanol, isobutanol, isopentanol, p-methoxy cinamyl alcohol, isopropanol, cyclohexanol, 1-phenyl ethanol, 1,5'-pentanediol, 1,6-hexanediol, 1,7-heptanediol, 1,8-octanediol, 1,9-nonanediol, salicyl alcohol and 4-nitrophenol	β -Glucosidase from almonds	As nonionic surfactants, in detergents and cosmetics	Katusumi et al. (2004)
(2) β -D-Glucopyranosides of propanol, hexanol and octanol	Raw almond meal	In detergents and cosmetics	Chahid et al. (1992, 1994)
(3) α/β -Glucopyranosides of ethanol, 1-propanol, 2-propanol, 2-methyl 2-propanol, 1-butanol, 2-butanol, 1-pentanol, 1-hexanol, 1,3-butanediol, 1,4-butanediol, 2,3-butanediol, 1,2-pentanediol, 1,5-pentanediol	Glucosylase and β -glucosidase	In detergents and cosmetics	Laroute and Willemot (1992)
(4) Allyl and benzyl β -D-glucopyranoside, allyl- β -D-galactopyranoside	Almond β -D-glucosidase	Used in the synthesis of glycopolymers, as temporary anomer-protected derivatives in carbohydrate chemistry	Vic and Crout (1995)
(5) n-Octyl glucoside, n-octyl galactoside	β -Galactosidase from <i>A. oryzae</i> , almond meal	In detergents and cosmetics	Chahid et al. (1994)
(6) n-Octyl - β -D-glucoside, 2-hydroxy benzyl glucopyranoside.	Almond β -glucosidase	In detergents and cosmetics	Vic et al. (1997)
(7) n-Octyl- β -D-glucoside, n-octyl- β -D-xylobioside, n-octyl- β -D-xyloside		As biological detergents and as emulsifying agents in cosmetics	Nakamura et al. (2000)
<i>B. Phenolic glycosides</i>			
(1) Eugenol- α -glucoside	α -Glucosyl transfer enzyme of <i>Xanthomonas campestris</i> WU-9701	As a prodrug of a hair restorer, as a derivative of spices	Sato et al. (2003)
2. Eugenol- β -glucoside	Biotransformation by cultured cells of <i>Eucalyptus perriniana</i>	As a prodrug of a hair restorer	Orihara et al. (1992)
(3) Vanillin- β -D-monoglucopyranoside	By suspension-cultured cells of <i>Coffea arabica</i>	As a food additive flavour	Kometani et al. (1993a)

(4) Capsaicin- β -D-glucopyranoside	By suspension-cultured cells of <i>Coffea arabica</i> By cultured cells of <i>Phytolacca americana</i>	Food ingredient and pharmacological applications	Kometani et al. (1993b) Hamada et al. (2003)
(5) α -Salicin, α -isosalicin, β -salicin	<i>Bacillus macerans</i> cyclodextrin glucanyl transferase and <i>Leuconostoc mesenteroides</i> B-742CB dextranase	Anti-inflammatory, analgesic antipyretic prodrug	Yoon et al. (2004)
(6) Curcumin glycosides	By cell suspension cultures of <i>Catharanthus roseus</i>	Food colourant, as antioxidant	Kaminaga et al. (2003)
<i>C. Flavonoid glycosides</i>			
(1) Quercetin-3-O- β -D-xylopyranosyl (1 \rightarrow 2)- β -D-galactopyranoside	Isolated from <i>Trifolium repens</i> L	UV-B radiation protection	Hofmann et al. (2000)
(2) Kaempferol-3-O- β -D-xylopyranosyl (1 \rightarrow 2)- β -D-galactopyranoside			
<i>D. Sweetener glycosides</i>			
(1) Stevioside, steviobioside, rebaudioside A, rebaudioside B	Isolated from the leaves of <i>Stevia rebaudiana</i>	As a natural sweetener, utilised in beverages	Kohda et al. (1976)
(2) Steviol-13-O-glucopyranoside, steviobioside, stevioside and rebaudioside	Enzyme fractions prepared from the soluble extracts of stevia	As a natural food sweeteners	Shibata et al. (1991)
<i>E. Terpinyl glycosides</i>			
(1) Geraniol β -glucoside, nerol β -glucoside, citroniol β -glucoside	β -Glucosidase from <i>A. niger</i> , <i>Trichoderma reesei</i> , <i>Candida molischiana</i> and almond	Good bioavailability, antifungal and antimicrobial activity	Gunata et al. (1994)
(2) Geraniol β -galactoside, nerol β -galactoside, citroniol β -galactoside	β -Galactosidase from <i>A. oryzae</i>	Good bioavailability, antifungal and antimicrobial activity	Donho et al. (1996)
<i>F. Glycosides in medicine</i>			
(1) Enediyne antibiotics – calicheamicin	Isolated from the cultivation broth of <i>Micromonospora echinospora</i>	Antitumor agents	Lee et al. (1987), Golik et al. (1987)
(2) Vitamin glycosides			
5'-O-(β -D-galactopyranosyl)-thiamin	<i>A. oryzae</i> β -galactosidase	Excellent nutritional efficiencies, more stable against UV and light.	Suzuki and Uchida (1994)

(continued)

Table 2.1 (continued)

Name of the compound	Source of enzyme	Applications	References
5'-O-(β -D-glucopyranosyl)-thiamin	Cyclomaltodextrin glucanotransferase from <i>Bacillus stearothermophilus</i>	Pleasant taste and odour, good bioavailability. More stable towards oxidative stress and UV irradiation	Uchida and Suzuki (1998)
4- α -D-glucopyranosyl rutin 2-O- α -glucopyranosyl-L-ascorbic acid	Cyclomaltodextrin glucanotransferase from <i>Bacillus stearothermophilus</i>		Suzuki and Suzuki (1991), Aga et al. (1990)
Alkaloid glycosides – elymoclavine-O- β -D-fructofuranoside	Isolated from a saprophytic culture of <i>Claviceps</i> sp.	In the treatment orthostatic circulatory disturbances, hypertension, hyperprolactinaemia, antibacterial and cytostatic effects and hypolipaeamic activity	Kren and Cvak (1999)
Steroidal glycosides – glycosides of diosgenin, solasodine, solasonine	Isolated from <i>Solanum</i> sp.	Anticarcinogenic activity	Nakamura et al. (1996)

References

- Aga H, Yoneyama M, Sakai S, Yamamoto I (1990) Synthesis of 2-O- α -D-glucopyranosyl L-ascorbic acid by cyclomaltodextrin glucotransferase from *Bacillus stearothermophilus*. *Agric Biol Chem* 55:1751–1756
- Akao T, Yoshino T, Kobashi K, Hatlori M (2002) Evaluation of salicin as an antipyretic prodrug that does not cause gastric injury. *Planta Med* 68:714–718
- Aleshin AE, Firsov LM, Honzatko RB (1994) Refined structure for the complex of acarbose with glucoamylases from *Aspergillus awamori* var. X100 to 2.4 Å resolution. *J Biol Chem* 269:15631–15639
- Ali S, Hossain Z (1991) Characteristics of glucoamylase from *Aspergillus terreus*. *J Appl Bacteriol* 71:144–146
- Ashikari T, Nakamura N, Tanaka Y, Kiuchi N, Shibano Y, Tanaka T, Amachi T, Yoshizumi H (1986) *Rhizopus* raw-starch-degrading glucoamylase Its cloning and expression in yeast. *Agric Biol Chem* 50:957–964
- Auge C, Fernandez RF, Gautheron CM (1990) The use of immobilized glycosyltransferases in the synthesis of sialyl oligosaccharides. *Carbohydr Res* 200:257–268
- Balzar D (1991) Alkylglucosides, their physico-chemical properties and their uses. *Tenside Surf Det* 28:419–427
- Bender H (1981) A bacterial glucoamylase degrading cyclodextrins. *Eur J Biochem* 115:287–291
- Berfoldo C, Anthranikian G (2001) Amyolytic enzymes from hyperthermophiles. *Methods Enzymol* 330:269–289
- Braun H, Cogoli A, Semenza G (1977) Carboxyl groups at the two active centers of sucrose-isomaltase from rabbit small intestine. *Eur J Biochem* 73:437–442
- Busch P, Hensen H, Khare J, Tesmann H (1994) Alkylpolyglycosides—a new cosmetic concept for mildness. *Agro-Food-Ind Hi-Tech* 5:20–28
- Chaga G, Porath J, Illeni T (1993) Isolation and purification of amyloglucosidase from *Halobacterium sodomense*. *Biomed Chromatogr* 7:256–261
- Chahid Z, Montet D, Pina M, Graille J (1992) Effect of water activity on enzymatic synthesis of alkylglycosides. *Biotechnol Lett* 14(4):281–284
- Chahid Z, Montet D, Pina M, Bonnot F, Graille J (1994) Biocatalyzed octylglycoside synthesis from a disaccharide. *Biotechnol Lett* 16:795–800
- Chiba S (1995) In: The Amylase Research Society of Japan (ed) *Enzyme chemistry and molecular biology of amylase and related enzymes*. CRC Press, Boca Raton/Ann arbor/London/Tokyo, pp 68–82
- Chiba S (1997) Molecular mechanism in α -glucosidase and glucoamylase. *Biosci Biotech Biochem* 61:1233–1239
- Chojceki A, Blaschek HP (1986) Effect of carbohydrate source on alpha-amylase and glucoamylase formation by *Clostridium acetobutylicum* SA-1. *Ind Microbiol* 1:63–67
- Clarks AJ, Svensson B (1984) Identification of an essential tryptophanyl residue in the primary structure of glucoamylase G2 from *Aspergillus niger*. *Carlsberg Res Commun* 49:559–566
- Crout DHG, Vic G (1998) Glycosidases and glycosyl transferases in glycoside and oligosaccharides synthesis. *Biocatal Biotransform* 2:98–111
- Czjzek M, Cicek M, Zamboni V, Bevan DR, Henrissat B, Esen A (2001) The mechanism of substrate (aglcone) specificity in β -glucosidase –DIMBOA, – DIMBOA Glc and –dhurrin complexes. *Proc Natl Acad Sci USA* 97:13555–13560
- Davies G, Henrissat B (1995) Structures and mechanisms of glycosyl hydrolases. *Structure* 3:853–859
- Donho M, Kimura T, Hara H (1996) Methods of producing geranyl β -D-galactopyranoside as flavoring material by enzymatic galactosylation of citronellol. *Jpn Kokai Tokkyo Koho JP* 8188589–8188591 (CA 125 222344)
- Ducret A, Carriere JF, Trani M, Lortie R (2002) Enzymatic synthesis of octyl glucoside catalysed by almond β -glucosidase in organic media. *Can J Chem* 80:653–656
- Ermer J, Rose K, Huber G, Schellenenberger A (1993) Subsite affinities of *Aspergillus niger* glucoamylase II determined with *p*-nitrophenylmaltooligosaccharides. *Biol Chem Hoppe Seyler* 374:123–128
- Ernst B, Hart GW, Sinay P (2000) *Carbohydrates in chemistry and biology*, vol 1. Wiley-VCH, Weinheim, pp 177–193
- Eveleigh DE, Perlin AS (1969) A proton magnetic resonance study of the anomeric species produced by D-glucosidases. *Carbohydr Res* 10:87–95
- Fagerstrom R (1991) Subsite mapping of *Hormoconis resinae* glucoamylase and their inhibition by gluconolactone. *J Gen Microbiol* 137:1001–1008
- Fischer E (1894) Einfluss der konfiguration auf die wirkung der enzyme. *Ber Chem Ges* 27:2985–2993
- Fogarty WM (ed) (1983) *Microbial amylases*. Microbial enzymes and biotechnology. Appl Science Publishers, London, pp 1–92
- Frandsen TP, Dupont C, Lehmbeck J, Stoffer B, Sierks MR, Honzatko RB, Svensson B (1994) Site-directed mutagenesis of the catalytic base Glutamic acid 400 in glucoamylase from *Aspergillus niger* and of Tyrosine 48 and Glutamine 401, both hydrogen bonded to the gamma-carboxylate group of Glutamic acid 400. *Biochemistry* 33:13808–13816
- Gellissen G, Janowicz ZA, Merkelbach A, Piontek M, Keup P, Weydemann U, Hollenberg CP, Sraßer AWM (1991) Heterologous gene expression in *Hansenula polymorpha*: efficient secretion of glucoamylase. *Biotechnology* 9:291–295
- Ghosh A, Chatterjee BS, Das A (1990) Characterization of glucoamylase from *Aspergillus terreus* 4. *FEMS Microbiol Lett* 66:345–349
- Golik J, Clardy J, Dubay G, Groenewold G, Kawaguchi H, Konishi M, Krishnan B, Ohkuma H, Saitoh K, Doby TW (1987) Esperamicins, a novel class of potent anti-tumor antibiotics. 3. Structures of esperamicins A1, A2 and A1b. *J Am Chem Soc* 109:3461–3464
- Gomes DCF, Alegrio LV, Leon LL, de Lima MEF (2002) Total synthesis and anti-leishmanial activity of some curcumin analogues. *Arzneim-Forsch* 52:695–698
- Gunata Z, Vallier MJ, Sapis JC, Baumes R, Bayonove C (1994) Enzymic synthesis of monoterpeny β -D-glucosides by various β -glucosidases. *Enzyme Microb Technol* 16:1055–1058

- Gygax D, Spies P, Winkler T, Pfaar U (1991) Enzymatic synthesis of β -D-glucuronides with *in situ* regeneration of uridine 5'-diphosphoglucuronic acid. *Tetrahedron* 47:5119–5122
- Haasum I, Ericksen SH, Jensen B, Olsen J (1991) Growth and glucoamylase production by the thermophilic fungus *Thermophilus lanuginose* in a synthetic medium. *Appl Microbiol Biotechnol* 34:656–660
- Hamada H, Nishida K, Furuya T, Ishihara K, Nakajima N (2003) Preparation of a new pepper: chemoenzymatic synthesis of capsaicin oligosaccharide and 8-nordihydrocapsaicin. *J Mol Catal B: Enzym* 16:115–119
- Harris EMS, Aleshin AE, Firsov LM, Honzatko RB (1993) Refined structure of the complex of 1-deoxynojirimycin with glucoamylase from *Aspergillus awamori* var X100. *Biochemistry* 32:1618–1626
- Hays WS, Vander Jagt DJ, Bose B, Serianni AS, Glew RH (1998) Catalytic mechanism and specificity for hydrolysis and transglycosylation reactions of cytosolic β -glucosidase from guinea pig liver. *J Biol Chem* 273:34941–34948
- He S, Withers SG (1997) Assignment of sweet almond β -glucosidase as a family I glycosidase and identification of its active site nucleophile. *J Biol Chem* 272:24864–24867
- Hiroimi K, Kawai M, Ono S (1966a) Kinetic studies on glucoamylase IV. Hydrolysis of isomaltose. *J Biochem* 59:476–480
- Hiroimi K, Takahashi K, Hamazu Z, Ono S (1966b) Kinetic studies on glucoamylase III. The influence of pH on the rates of hydrolysis of maltose and panose. *J Biochem* 59:469–475
- Hiroimi K, Nitta Y, Numata C, Ono S (1973) Subsite affinities if glucoamylase examination of the validity of the subsite theory. *Biochem Biophys Acta* 302:362–375
- Hofmann RW, Swinny EE, Bloor SJ, Markham KR, Ryan KG, Campbell BD, Jordan BR, Fountain DW (2000) Responses of nine *Trifolium repens* L. populations to ultraviolet-B radiation. Differential flavonol glycoside accumulation and biomass production. *Ann Bot* 86:527–537
- Hyun HH, Zeikus JG (1985) General biochemical characterization of thermostable pullulanase and glucoamylase from *Clostridium thermohydrosulfuricum*. *Appl Environ Microbiol* 49:1168–1173
- Igarashi K (1977) The Koenigs-Knorr reaction. *Adv Carbohydr Chem Biochem* 34:243–283
- Ikeda D, Umezawa S (1999) Aminoglycoside antibiotics. In: Ikan R (ed) *Naturally occurring glycosides*. Wiley, England, pp 1–42
- Ismail A, Linder M, Ghoul M (1999a) Optimization of butylgalactoside synthesis by β -galactosidase from *Aspergillus oryzae*. *Enzyme Microb Technol* 25:208–213
- Ismail A, Soutani S, Ghoul M (1999b) Enzymatic-catalyzed synthesis of alkylglycosides in monophasic and biphasic systems. I. The transglycosylation reaction. *J Biotechnol* 69:135–143
- Itoh T, Sakata Y, Akada R, Nimi O, Yamshita I (1989) Construction and characterization of mutant glucoamylases from the yeast *Saccharomycopsis fibuligera*. *Agric Biol Chem* 53:3159–3168
- IUBMB (1992) *Enzyme nomenclature*. Academic Press, San Diego, California, ISBN 0-12-227164-5
- Jacobson RH, Zhang X-J, DuBose RF, Matthews BW (1994) Three dimensional structure of β -galactosidase from *E. Coli*. *Nature* 369:761–766
- Jacobson RH, Kuroki R, Weaver LH, Zhang X-J, Matthews BW (1995) In: Saddler JN, Penner MH (eds) *Enzymatic degradation of insoluble carbohydrates*, vol 618. ACS Symposium Series, Washington, DC, pp 38–50
- James JA, Lee BH (1995) Cultural conditions for production of glucoamylase from *Lactobacillus amylovorus* ATCC 33621. *J Appl Bacteriol* 79:499–505
- Kaljuzhin OV, Shkalev MV (2000) Immunomodulator and pharmaceutical compositions with antitumor properties, and a food additive. Patent EP1038532 (CA 129 335732)
- Kaminaga Y, Nagatsu A, Akiyama T, Sugimoto N, Yamazaki T, Maitani T, Mizukami H (2003) Production of unnatural glucosides of curcumin with drastically enhanced water solubility by cell suspension cultures of *Catharanthus roseus*. *FEBS Lett* 555:311–316
- Kaper T, Lebbink JHG, Pouwels J, Kopp J, Schulz GE, Oost JV, Vos WM (2000) Comparative structural analysis and substrate specificity engineering of the hyperthermostable β -glucosidase CelB from *Pyrococcus furiosus*. *Biochemistry* 39:4963–4970
- Katusumi K, Mikio F, Yoshiteru I, Hiroyuki A (2004) Simple synthesis of β -D-glycopyranosides using β -glucosidase from almonds. *Chem Pharm Bull* 52:270–275
- Kengen SWM, Luesink EJ, Stams AJM, Zehnder AJB (1993) Purification and characterization of an extremely thermostable β -glucosidase from the hyperthermophilic archaeon *Pyrococcus furiosus*. *Eur J Biochem* 213:305–312
- Kleinman MJ, Wilkinson AE, Wright IP, Evans IH, Bevan EA (1988) Purification and properties of an extracellular glucoamylase from a diastatic strain of *Saccharomyces cerevisiae*. *Biochem J* 249:163–170
- Kohda H, Kasai R, Yamasaki K, Tanaka O (1976) New sweet diterpene glucosides from *Stevia rebaudiana*. *Phytochemistry* 15:981–983
- Kojima M, Maruo S, Ohgi T, Ezure Y (1996) Enzymatic synthesis of 4-O- β -D-galactopyranosylmoranoline and 3-O- β -D-galactopyranosylmoranoline by using β -galactosidase from *Bacillus circulans*. *Biosci Biotech Biochem* 60:694–696
- Kometani T, Tanimoto H, Nishimura T, Kanbara I, Okada S (1993a) Glucosylation of capsaicin by cell suspension cultures of *Coffea arabica*. *Biosci Biotech Biochem* 57:2192–2193
- Kometani T, Tanimoto H, Nishimura T, Okada S (1993b) Glucosylation of vanillin by cultured plant cells. *Biosci Biotech Biochem* 57:1290–1293
- Konstantinovic S, Predojevic J, Gojkovic S, Ratkovic Z, Mojsilovic B, Pavlovic V (2001) Synthesis of C7-C16 alkyl 2,3 dideoxy glucosides from glucose and fatty acids. *Ind J Chem* 40B:1242–1244
- Krause DR, Wood CJ, MacLean DJ (1991) Glucoamylase (exo-1,4- α -D-glucohydrolase, E.C. 3.2.1.3) is the

- major starch-degrading enzyme secreted by the phytopathogenic fungus *Colletotrichum gloeosporioides*. *J Gen Microbiol* 137:2463–2468
- Kren V (2001) Chemical biology and biomedicine of glycosylated natural compounds. In: Fraser-Reid B, Tatsuta K, Thiem J (eds) *Glycoscience chemistry and chemical biology*, vol 3. Springer, Berlin, pp 2471–2529
- Kren V, Cvak L (1999) Ergot genus *Claviceps*, medicinal and aromatic plants-industrial profiles. Harwood Publ. Ltd., Amsterdam/London
- Kren V, Martinkova L (2001) Glycosides in medicine: the role of glycosidic residue in biological activity. *Curr Med Chem* 8:1313–1338
- Laroute V, Willemot RM (1992) Glucoside synthesis by glucoamylase or β -glucosidase in organic solvents. *Biotechnol Lett* 14:169–174
- Lee MD, Dunne TS, Chang CC, Ellestad GA, Siegel MM, Morton GO, McGahren WJ, Borders DB (1987) Calicheimicines, a novel family of antitumor antibiotics 2. Chemistry and structure of calicheimicin, γ I. *J Am Chem Soc* 109:3466–3468
- Lehinger AL (1975) Sugars, storage polysaccharides and cell walls. In: *Biochemistry*. Worth Publishers Inc., New York, pp 249–276
- Ljunger G, Adlercreutz P, Mattiasson B (1994) Enzymatic synthesis of octyl- β -glucoside in octanol at controlled water activity. *Enzyme Microb Technol* 16:751–755
- Madsen T, Petersen G, Seiero C, Torslov J (1996) Biodegradability and aquatic toxicity of glycoside surfactants and a nonionic alcohol etherate. *J Am Oil Chem Soc* 73:929–933
- Malek SAS, Hossain Z (1994) Purification and characterization of a thermostable glucoamylase from *Myrothecium* isolate. *J Appl Bacteriol* 76:210–215
- Matsumura Y, Kasunoki M, Harada W, Kakudo M (1984) Structure and possible catalytic residues of taka amylase A. *J Biochem* 95:697–702
- Matsumura S, Imai K, Yoshikawa S, Kawada K, Uchibori T (1990) Surface activities, biodegradability and antimicrobial properties of n-alkyl glucosides, manosides and galactosides. *J Am Oil Chem Soc* 67:996–1001
- McCarter J, Withers SG (1994) Mechanisms of enzymatic glycoside hydrolysis. *Curr Opin Struct Biol* 4:885–892
- Mohri K, Watanabe Y, Yoshida Y, Satoh M, Isobe K, Sugimoto N, Tsuda Y (2003) Synthesis of glycosyl-curcuminoids. *Chem Pharm Bull* 51:1268–1272
- Monsan PF, Paul F, Pelenc P, Boulter E (1996) Enzymatic production of α -butyl glucoside and its fatty acid esters. *Ann NY Acad Sci* 799:633–641
- Mutua LN, Akoh CC (1993) Synthesis of alkyl glucoside fatty acid esters in non aqueous media by *Candida* sp. lipase. *J Am Oil Chem Soc* 70:43–46
- Nakamura T, Komori C, Lee Y-Y, Hashimoto F, Yohara S, Nohara T, Ejima A (1996) Cytotoxic activities of solanum steroidal glycosides. *Biol Pharm Bull* 19:546–566
- Nakamura T, Toshima K, Matsumura S (2000) One-step synthesis of n-octyl β -D-xylotrioside, xylobioside and xyloside from xylan and n-octanol using acetone powder of *Aureobasidium pullulans* in supercritical fluids. *Biotechnol Lett* 22:1183–1189
- Nilsson KGI (1987) A simple strategy for changing the regio selectivity of glycosidase catalyzed formation of disaccharides. *Carbohydr Res* 167:95–103
- Ohinishi M (1990) Subsite structure of *Rhizopus niveus* glucoamylase, estimated with the binding parameters for maltooligosaccharides. *Starch/Stärke* 42:311–313
- Ohinishi H, Sakai H, Ohta T (1991) Purification and some properties of a glucoamylase from *Clostridium* sp. G0005. *Agric Biol Chem* 55:1901–1902
- Okada G, Unno T (1989) A glucodextranase accompanied by glucoamylase activity from *Arthrobacter globiformis* I 42. *Agric Biol Chem* 53:223–228
- Ooi Y, Hashimoto T, Mitsuo N, Satoh T (1985) Enzymatic formation of β -galactosidase from *Aspergillus oryzae* and its application to the synthesis of chemically unstable cardiac glycosides. *Chem Pharm Bull* 33:1808–1814
- Orihara Y, Furuya T, Hashimoto N, Deguchi Y, Tokoro K, Kanisawa T (1992) Biotransformation of isoeugenol and eugenol by cultured cells of *Eucalyptus perriniana*. *Phytochemistry* 31:827–831
- Panintrarux C, Adachi S, Araki Y, Kimura Y, Matsuno R (1995) Equilibrium yield of n-alkyl- β -D-glucoside through condensation of glucose and n-octanol by β -galactosidase in a biphasic system. *Enzyme Microb Technol* 17:32–40
- Payen A, Persoz JF (1833) Mémoire sur la diastase, les principaux produits de ses reactions et leur applications aux arts industriels. *Annales de chimie et de physique* 53:73–92
- Post CB, Karplus M (1986) Does lusozye follow the lusozye pathway? An alternative based on dynamic structural and stereoelectronic considerations. *J Am Chem Soc* 108:1317–1319
- Pretorius IS, Lambrechts MG, Marmur J (1991) The glucoamylase multigene family in *Saccharomyces cerevisiae* var. *diastaticus* an overview. *CRC Crit Rev Biochem Mol Biol* 26:53–76
- Pugh TA, Shah JC, Magee PT, Clancy MJ (1989) Characterization and localization of the sporulation glucoamylase from *Saccharomyces cerevisiae*. *Biochem Biophys Acta* 994:200–209
- Rantwijk FV, Oosterom MW, Sheldon RA (1999) Glycosidase-catalyzed synthesis of alkyl glycosides. *J Mol Catal B: Enzym* 6:511–532
- Rao VB, Sastri NVS, Rao PVS (1981) Purification and characterization of a thermostable glucoamylase from the thermophilic fungus *Thermomyces lanuginose*. *Biochem J* 193:379–385
- Robyt JF (1998) *Essentials of carbohydrate chemistry*. Springer, New York, pp 64–68
- Roitsch T, Lehle L (1989) Structural requirements for protein N-glycosylation. Influence of acceptor peptides on cotranslational glycosylation of yeast influence and site-directed mutagenesis around a sequon sequence. *Eur J Biochem* 181:525–529
- Rubio E, Fernandez MA, Klivanov AM (1991) Effect of the solvent on enzyme regio selectivity. *J Am Chem Soc* 113:695–696

- Saha BC, Zeikus JG (1989) Microbial glucoamylases biochemical and biotechnological features. *Starch/Starke* 41:57–64
- Sakata I, Maruyama I, Kobayashi A, Yamamoto I (1998) Production of phenethyl alcohol glycoside. *Jpn Kokai Tokkyo Koho, Japan Patent JP 10052297* (CA 128 229438)
- San-Aparicio J, Hermoso JA, Martinz-Ripoll M, Laquerica JL, Polaina J (1998) Crystal structure of β -glucosidase A from *Bacillus polymyxa* insights into the catalytic activity in family I glycosyl hydrolases. *J Mol Biol* 275:491–502
- Sato T, Takeuchi H, Takahashi K, Kurosu J, Yoshida K, Tsugane T, Shimura S, Kino K, Kirimura K (2003) Selective α -glucosylation of eugenol by α -glucosyl transfer enzyme of *Xanthomonas campestris* WU-9701. *J Biosci Bioeng* 96:199–202
- Schmid B, Kotter I, Heide L (2001) Pharmacokinetics of salicin after oral administration of a standard willow bark extract. *Eur J Clin Pharmacol* 57:387–391
- Shibata H, Sonoke S, Ochiai H, Nishihashi H, Yamada M (1991) Glucosylation of steviol and steviol glucosides in extracts from *Stevia rebaudiana* Bertoni. *Plant Physiol* 95:152–156
- Shin HK, Kong JY, Lee JD, Lee TH (2000) Synthesis of hydroxy benzyl- α -glucosides by amyloglucosidase-catalysed transglycosylation. *Biotechnol Lett* 22:321–325
- Shinoyama H, Kamiyama Y, Yasui T (1988) Enzymatic synthesis of alkyl β -xylosides from xylobiose by application of the transxylosyl reaction of *Aspergillus niger* β -xylosidase. *Agric Biol Chem* 52:2197–2202
- Sierks MR, Ford C, Reilly PJ, Svensson B (1990) Catalytic mechanism of fungal glucoamylases as defined by mutagenesis of Asp 176, Glu179, and Glu180 in the enzyme from *Aspergillus awamori*. *Protein Eng* 3:193–198
- Sills AM, Saunder ME, Stewart GG (1984) Isolation and characterization of the amylolytic system of *Schwanniomyces castellii*. *J Inst Brew* 90:311–316
- Sinnot ML (1990) Catalytic mechanism of glycosyl transfer. *Chem Rev* 90:1171–1202
- Sivakumar R (2009) Enzymatic synthesis of selected phenolic and vitamin glycosides. PhD thesis, University of Mysore
- Soni BK, Kapp C, Goma G, Soucaille P (1992) Solvent production from starch effect of pH on α -amylase and glucoamylase localization and synthesis in synthetic medium. *Appl Microbiol Biotechnol* 37:539–543
- Specka U, Mayer F, Antranikian G (1991) Purification and properties of thermoactive glucoamylase from *Clostridium thermosaccharolyticum*. *Appl Environ Microbiol* 57:2317–2323
- Srivastava RAK (1984) Studies on extracellular and intracellular purified amylases from a thermophilic *Bacillus stearothermophilus*. *Enzyme Microb Technol* 6:422–426
- Stevenson DE, Furneaux RH (1996) High yield synthesis of ethyl and 2-fluoroethyl β -D-galactopyranosides using *Streptococcus thermophilus* β -galactosidase. *Enzyme Microb Technol* 18:513–518
- Stevenson DE, Stanley RA, Furneaux RH (1993) Optimization of alkyl β -D-galactopyranoside synthesis from lactose using commercially available β -galactosidase. *Biotechnol Bioeng* 42:657–666
- Stoffer B, Frandsen T, Busk P, Schneider P, Svendsen I, Svensson B (1993) Production, purification and characterization of the catalytic domain of glucoamylase from *Aspergillus niger*. *Biochem J* 292:197–202
- Stoffer B, Aleshin AE, Firsov LM, Svensson B, Honzatko RB (1995) Refined structure for the complex of D-glucosyl-dihydroacarbose with glucoamylases from *Aspergillus awamori* var. X100 to 2.2 Å resolution dual conformation for extended inhibitors bound to the active site of glucoamylases. *FEBS Lett* 358:57–61
- Suzuki Y, Suzuki K (1991) Enzymatic formation of 4 G- α -D-glucopyranosyl rutin. *Agric Biol Chem* 55:181–187
- Suzuki Y, Uchida K (1994) Enzymatic formation of a new derivative of thiamin, β -galactosylthiamin. *Biosci Biotech Biochem* 58:1273–1276
- Svensson B, Larsen K, Svendsen I, Boel E (1983) The complete amino acid sequence of the glycoprotein glucoamylase G1 from *Aspergillus niger*. *Carlsberg Res Commun* 48:529–544
- Svensson B, Clarke AJ, Svendsen I, Moller H (1990) Identification of carboxylic acid residues in glucoamylase G2 from *Aspergillus niger* that participate in the catalysis and substrate binding. *Eur J Biochem* 18:29–38
- Takahashi T, Kato K, Ikegami Y, Irie M (1985) Different behavior towards raw starch of three forms of glucoamylase from a *Rhizopus* sp. *J Biochem* 98:663–671
- Tanaka Y, Ashikari T, Nakamura N, Kiuchi N, Shibano Y, Amachi T, Yoshizumi H (1986) Comparison of amino acid sequences of three glucoamylases and their structure-function relationships. *Agric Biol Chem* 50:965–969
- Tapavicza SV, Bell D, Kopp-Holtwiesche B (2000) Plant growth enhancement against phytopathogenic fungi and/or soil borne pests. Patent WO 0002451 (CA 132 60488)
- Taylor PM, Napier EJ, Fleming ID (1978) Some properties of a glucoamylase produced by the thermophilic fungus *Humicola lanuginosa*. *Carbohydr Res* 16:301–308
- Trincon A, Pagnotta E, Giordano A, Perugino G, Rossi M, Moracci M (2003) Enzymatic synthesis of 2-deoxyglycosides using the β -glycosidase of the archaeon *Sulfolobus solfataricus*. *Biocatal Biotransform* 21:17–24
- Uchida K, Suzuki Y (1998) Enzymatic synthesis of a new derivative of thiamin, O- α -glucosylthiamin. *Biosci Biotech Biochem* 62(2):221–224
- Verdoucq L, Czjzek M, Moriniere J, Beven DR, Esen A (2003) Mutational and structural analysis of aglycone specificity in maize and sorghum β -glucosidase. *J Biol Chem* 278:25055–25062
- Vic G, Crout DHG (1995) Synthesis of allyl and benzyl β -D-glucopyranosides and allyl β -D-galactopyranoside from D-glucose or D-galactose and the corresponding

- alcohol using almond β -D-glucosidase. *Carbohydr Res* 279:315–319
- Vic G, Thomas D (1992) Enzyme-catalyzed synthesis of alkyl- β -D-glucosides in organic media. *Tetrahedron Lett* 33:4567–4570
- Vic G, Biton J, Beller DL, Michel JM, Thomas D (1995) Enzymatic glycosylation of hydrolytic alcohols in organic medium by the reverse hydrolysis reaction using almond β -D-glucosidase. *Biotechnol Bioeng* 46:109–116
- Vic G, Thomas D, Crout DHG (1997) Solvent effect on enzyme-catalyzed synthesis of β -D-glucosides using the reverse hydrolysis method application to the preparative-scale synthesis of 2-hydroxybenzyl and octyl β -D-glucopyranosides. *Enzyme Microb Technol* 20:597–603
- Vijayakumar GR (2007) Enzymatic synthesis of selected glycosides. PhD thesis, University of Mysore
- Voorhorst WGB, Eggen RIK, Luesink EJ, De Vos WM (1995) Characterization of the Cel B gene coding for β -glucosidase from the hyperthermophilic archaeon *Pyrococcus furiosus* and its expression and site directed mutation in *Escherichia coli*. *J Bacteriol* 177:7105–7111
- Vulfson EN, Patel R, Beecher JE, Andrews AT, Law BA (1990) Glycosidases in organic solvents I. Alkyl- β -glucoside synthesis in a water-organic two-phase system. *Enzyme Microb Technol* 12:950–954
- Williamson G, Belshaw NJ, Williamson MP (1992) O-Glycosylation in *Aspergillus* glucoamylase. Confirmation and role in binding. *Biochem J* 282:423–428
- Withers SG, Street IP (1988) Identification of a covalent α -D-glucopyranosyl enzyme intermediate formed on a β -glucosidase. *J Am Chem Soc* 110:8551–8553
- Withers GG, Warren RAJ, Street IP, Rupitz K, Kempton JB, Abersold R (1990) Unequivocal demonstration of the involvement in the mechanism of a retaining glycosidase. *J Am Chem Soc* 112:5887–5889
- Yoon SH, Fulton DB, Robyt JF (2004) Enzymatic synthesis of two salicin analogues by reaction of salicyl alcohol with *Bacillus macerans* cyclomaltodextrin glucanyltransferase and *Leuconostoc mesenteroides* B-742CB dextranucrase. *Carbohydr Res* 339:1517–1529
- Yu RC, Hang YD (1991) Purification and characterization of a glucoamylase from *Rhizopus oryzae*. *Food Chem* 40:301–308
- Zechel DL, Withers SG (2001) Dissection of nucleophilic and acid–base catalysis in glycosidases. *Curr Opin Chem Biol* 5:643–649
- Zhou JH (2000) Herbal sweetening and preservative composition comprising licorice extract and mogrosides obtained from plants belonging to cucurbitaceae and/or momordica. Patent US 6103240 (CA 133 168393)



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