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-glucanohydralse; EC 3.2.1.1) is an endo-acting enzyme which hydrolysies 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 (β/α) 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 maltooligosaccharide or saccharogen amylose, 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 glucosidic 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 gloeosporiodes* (Krause et al. 1991).
2.6 Structural Features of Glucoamylase

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), *Saccharomyces 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. stea-thermophilus* (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* (Chojecki 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 archean *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 alkyla-
tion (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 glucosi-
dase 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 bind-
ing (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 cata-
lytic 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 struc-
ture 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 archean Pyrococcus furiosus, at a molecular level exhibiting a homotetramer configuration, with subunits having a typical (βα)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 glycosidases 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 (Trincone 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
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 anomic configuration of the substrate. The two catalytic ionisable groups, a carboxyl, –COOH, and a carboxylate, -COO$, cleave the glucosidic linkage cooperatively by direct electrophilic and nucleophilic attacks against the glycosyl oxygen and anomic 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 anomic configuration of the product by the double displacement. The double displacement mechanism is adequate for explaining the reaction, where the anomic 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 anomic 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
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 ( Sinnott 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
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 bioavailability 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.

Scheme 2.3  Oxo-carbenium ion intermediate mechanism (Chiba 1997)
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 desirable 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; Gygax 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>
<thead>
<tr>
<th>Name of the compound</th>
<th>Source of enzyme</th>
<th>Applications</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>A. Surfactant glycosides</strong></td>
<td></td>
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<tr>
<td>(1) α- and 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</td>
<td>β-Glucosidase from almonds</td>
<td>As nonionic surfactants, in detergents and cosmetics</td>
<td>Katusumi et al. (2004)</td>
</tr>
<tr>
<td>(2) β-D-Glucopyranosides of propanol, hexanol and octanol</td>
<td>Raw almond meal</td>
<td>In detergents and cosmetics</td>
<td>Chahid et al. (1992, 1994)</td>
</tr>
<tr>
<td>(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</td>
<td>Glucoamylase and β-glucosidase</td>
<td>In detergents and cosmetics</td>
<td>Laroute and Willemot (1992)</td>
</tr>
<tr>
<td>(4) Allyl and benzyl β-D-glucopyranoside, allyl-β-D-galactopyranoside</td>
<td>Almond β-D-glucosidase</td>
<td>Used in the synthesis of glycopolymers, as temporary anomeric-protected derivatives in carbohydrate chemistry</td>
<td>Vic and Crout (1995)</td>
</tr>
<tr>
<td>(5) n-Octyl glucoside, n-octyl galactoside</td>
<td>β-Galactosidase from A. oryzae, almond meal</td>
<td>In detergents and cosmetics</td>
<td>Chahid et al. (1994)</td>
</tr>
<tr>
<td>(6) n-Octyl-β-D-glucoside, 2-hydroxy benzyl glucopyranoside</td>
<td>Almond β-glucosidase</td>
<td>In detergents and cosmetics</td>
<td>Vic et al. (1997)</td>
</tr>
<tr>
<td>(7) n-Octyl-β-D-glucoside, n-octyl-β-D-xylobioside, n-octyl-β-D-xyloside</td>
<td></td>
<td></td>
<td>Nakamura et al. (2000)</td>
</tr>
<tr>
<td><strong>B. Phenolic glycosides</strong></td>
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</tr>
<tr>
<td>(1) Eugenol-α-glucoside</td>
<td>α-Glucosyl transfer enzyme of Xanthomonas campestris WU-9701</td>
<td>As a prodrug of a hair restorer, as a derivative of spices</td>
<td>Sato et al. (2003)</td>
</tr>
<tr>
<td>2. Eugenol-β-glucoside</td>
<td>Biotransformation by cultured cells of Eucalyptus perriniana</td>
<td>As a prodrug of a hair restorer</td>
<td>Orihara et al. (1992)</td>
</tr>
<tr>
<td>(3) Vanillin-β-D-monoglucopyranoside</td>
<td>By suspension-cultured cells of Coffea arabica</td>
<td>As a food additive flavour</td>
<td>Kometani et al. (1993a)</td>
</tr>
</tbody>
</table>
### Advantages of Enzymatic Glycosylation over Chemical Methods

<table>
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<th>Name of the compound</th>
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<tr>
<td>Capsaicin-β-D-glucopyranoside</td>
<td>By suspension-cultured cells of <em>Coffea arabica</em> By cultured cells of <em>Phytolacca americana</em></td>
<td>Food ingredient and pharmacological applications</td>
<td>Kometani et al. (1993b) Hamada et al. (2003)</td>
</tr>
<tr>
<td>α-Salicyc, α-isosalicyc, β-salicin</td>
<td><em>Bacillus macerans</em> cyclodextrin glucan transferase and <em>Leuconostoc mesenteroides</em> B-742CB dextranucrase</td>
<td>Anti-inflammatory, analgesic antipyretic prodrug</td>
<td>Yoon et al. (2004)</td>
</tr>
<tr>
<td>Curcumin glycosides</td>
<td>By cell suspension cultures of <em>Catharanthus roseus</em></td>
<td>Food colourant, as antioxidant</td>
<td>Kaminaga et al. (2003)</td>
</tr>
<tr>
<td>Quercetin-3-O-β-D-xylopyranosyl (1→2)-β-D-galactopyranoside</td>
<td>Isolated from <em>Trifolium repens L</em></td>
<td>UV-B radiation protection</td>
<td>Hofmann et al. (2000)</td>
</tr>
<tr>
<td>Kaempferol-3-O-β-D-xylopyranosyl (1→2)-β-D-galactopyranoside</td>
<td></td>
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<tr>
<td>Steviol glycosides</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Stevioside, steviolbioside, rebaudioside A, rebaudioside B</td>
<td>Isolated from the leaves of <em>Stevia rebaudiana</em></td>
<td>As a natural sweetener, utilised in beverages</td>
<td>Kohda et al. (1976)</td>
</tr>
<tr>
<td>Steviol-13-O-glucopyranoside, steviolbioside, stevioside and rebaudioside</td>
<td>Enzyme fractions prepared from the soluble extracts of stevia</td>
<td>As a natural food sweeteners</td>
<td>Shibata et al. (1991)</td>
</tr>
<tr>
<td>E. Terpinyl glycosides</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gereniol β-glucoside, nerol β-glucoside, citroniol β-glucoside</td>
<td>β-Glucosidase from <em>A. niger, Trichoderma reesei, Candida molischiana</em> and almond</td>
<td>Good bioavailability, antifungal and antimicrobial activity</td>
<td>Gunata et al. (1994)</td>
</tr>
<tr>
<td>Gereniol β-galactoside, nerol β-galactoside, citroniol β-galactoside</td>
<td>β-Galactosidase from <em>A. oryzae</em></td>
<td>Good bioavailability, antifungal and antimicrobial activity</td>
<td>Donho et al. (1996)</td>
</tr>
<tr>
<td>F. Glycosides in medicine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enediyne antibiotics – calicheamicin</td>
<td>Isolated from the cultivation broth of <em>Micromonospora echinospora</em></td>
<td>Antitumor agents</td>
<td>Lee et al. (1987), Golik et al. (1987)</td>
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<td>Vitamin glycosides</td>
<td></td>
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<tr>
<td>5'-O-(β-D-galactopyranosyl)-thiamin</td>
<td><em>A. oryzae</em> β-galactosidase</td>
<td>Excellent nutritional efficiencies, more stable against UV and light.</td>
<td>Suzuki and Uchida (1994)</td>
</tr>
<tr>
<td>Name of the compound</td>
<td>Source of enzyme</td>
<td>Applications</td>
<td>References</td>
</tr>
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<tr>
<td>5′-O-(β-D-glucopyranosyl)-thiamin</td>
<td>Cyclomaltodextrin glucanotransferase from <em>Bacillus</em> stearothermophilus</td>
<td>Pleasant taste and odour, good bioavailability. More stable towards oxidative stress and UV irradiation</td>
<td>Uchida and Suzuki (1998)</td>
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<tr>
<td>4-α-D-glucopyranosyl rutin</td>
<td>Cyclomaltodextrin glucanotransferase from <em>Bacillus</em> stearothermophilus</td>
<td></td>
<td>Suzuki and Suzuki (1991), Aga et al. (1990)</td>
</tr>
<tr>
<td>2-O-α-glucopyranosyl-β-l-ascorbic acid</td>
<td></td>
<td></td>
<td>Kren and Cvak (1999)</td>
</tr>
<tr>
<td>Alkaloid glycosides – elymoclavine-O-β-D-fructofuranoside</td>
<td>Isolated from a saprophytic culture of <em>Claviceps</em> sp.</td>
<td>In the treatment orthostatic circulatory disturbances, hypertension, hyperprolactinaemia, antibacterial and cytostatic effects and hypolipaemic activity</td>
<td>Kren and Cvak (1999)</td>
</tr>
<tr>
<td>Steroidal glycosides – glycosides of diosgenin, solasodine, solasonine</td>
<td>Isolated from <em>Solanum</em> sp.</td>
<td>Anticarcinogenic activity</td>
<td>Nakamura et al. (1996)</td>
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</tbody>
</table>
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Enzymatic Transformation
Divakar, S.
2013, XX, 284 p. 398 illus., 14 illus. in color., Hardcover
ISBN: 978-81-322-0872-3