CHAPTER I

INTRODUCTION

1.1 Starch

Starch is a major polysaccharide in plants such as rice, corn, potato, etc. Starch is synthesized in plastids: chloroplast, in leaves and amyloplast, in tuberous roots and seeds. Amylose and amylopectin are both components of starch. Amylose is a linear polymer of glucose residues with 100 up to 1 million glucose units (Manner, 1989) linked by α-1,4 glycosidic bond. Amylopectin, a branched polymer, consists of more than 200 millions of glucose units (Manner, 1989) connected linearly by an α-1,4 glycosidic bond while branch points are linked by an α-1,6 glycosidic bond, at every 24-30 glucose residues (Figure 1.1).

Most of plant starch consist of 20-30% amylose and 70-80% amylopectin, however, the ratio of these 2 components depends on types of plants (Table 1.1). In applications, starch is used as stabilizer, film forming, coating agent, thickener and emulsifier in food, and also used in pharmaceutical, cosmetic, textile and paper industries (Shama, 1981; Manek *et al.*, 2005; Glittenberg, 2012). For example, sago palm (*Metroxylon sagu*) starch is used as film matrix in food packaging (Bhat *et al.*, 2013), starch from wheat and oat are used to improve coating in paper industries (Wihelm, 1993), and corn starch is used as gel forming in drug delivery (Wierik *et al.*, 1997; Rahmouni *et al.*, 2003).
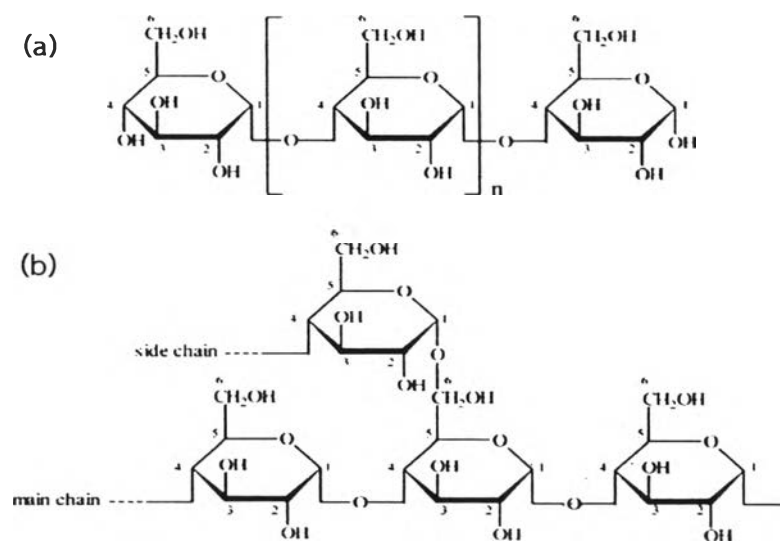
Figure 1.1   Starch components: amylose (a) and amylopectin (b) (Herrero-Mart'inez *et al.* 2004)

Table 1.1   Amylose content in different sources of starch (Kasemsuwan *et al.* 1999)

Starch	Apparent Amylose content (% dry weight)
Wheat	28.8
Maize	29.4
Rice	25.0
Barley	25.5
Potato	36.0
Tapioca	23.5
Pea	37.9

1.2 Amylases and 4α-glucanotransferase

The amylase family is a group of enzymes that act on starch by hydrolysis of glycosidic linkages of starch with different specificity to glucose polymer substrate (van der Maarel *et al.* 2002) such as glucose residues connected by α-1,1, α-1,4 or α-1,6 glycosidic bond as shown in Table 1.2.

Amylases can be classified into 4 groups. Firstly, endoamylases such as α-amylase, the enzyme hydrolyzing α-1,4 glycosidic bond from inner chain of amylose or amylopectin, producing branched oligosaccharides, linear oligosaccharides or limit dextrins. Secondly, exoamylases which catalyze the hydrolysis of α-1,4 or α-1,6 glycosidic linkage from non-reducing end of amylose and amylopectin results in short chain products. The enzymes in this group such as β-amylase, glucoamylase and amyloglucosidase (Pandey *et al.* 2000, Gupta *et al.* 2003). Thirdly, debranching enzymes such as isoamylase, pullulanase type I, hydrolyse α-1,6 glycosidic bond and give long chain oligosaccharides as products (Bender *et al.* 1995, Israilides *et al.* 1999). Finally, transferases which hydrolyse α-1,4 glycosidic linkage of donor and transfer to glycosidic acceptor so a new glycosidic bond is formed (Figure 1.2). This group is named 4α-glucanotransferase, consisting of amylomaltase, cyclodextrin glycosyltransferase (CGTase) and glycogen debranching enzyme (Takaha and Smith 1999, van der Veen *et al.* 2000).

4α-glucanotransferase catalyzes four different reactions (Figure 1.3). The first is disproportionation which is an intermolecular transglucosylation reaction. The enzyme transfers the glucosyl units from short chain oligosaccharide to the free OH group at C4 of other short chain oligosaccharides which results in oligosaccharides with various length as in the following equation:

Table 1.2   Main substrates of the enzyme in amylase family

(Reddy *et al.* 2003)

| Enzyme	EC number	Main substrate
Amylosucrase	EC: 2.4.1.4	Sucrose
Sucrose phosphorylase	EC: 2.4.1.7	Sucrose
Glucan branching enzyme	EC: 2.4.1.18	Starch, glycogen
Cyclomaltodextrin glycosyltransferase	EC: 2.4.1.9	Starch
Amylomaltase	EC: 2.4.1.25	Starch, glycogen
Maltopentaose-forming α-amylase	EC: 3.2.1.-	Starch
α-amylase	EC: 3.2.1.1	Starch
Oligo-1,6-glucosidase	EC: 3.2.1.10	1,6-α-D-glucosidic lingkage
α-glucosidase	EC: 3.2.1.20	Starch
Amylopullulanase	EC: 3.2.1.41	Pullulan
Cyclomaltodextrinase	EC: 3.2.1.54	Linear and cyclomaltodextrin
Isopullulanase	EC: 3.2.1.57	Pullulan
Maltotetraose-forming α-amylase	EC: 3.2.1.60	Starch
Isoamylase	EC: 3.2.1.68	Amylopectin
Glucodextranase	EC: 3.2.1.70	Starch
Tehalose-6-phosphate-hydrolase	EC: 3.2.1.93	Trehalose
Maltohexaose-forming α-amylase	EC: 3.2.1.98	Starch
Maltogenic amylase	EC: 3.2.1.133	Starch
Neopullulanase	EC: 3.2.1.135	Pullulan
Malto-oligosyl trehalase hydrolase	EC: 3.2.1.141	Trehalose
Malto-oligosyl trehalose synthase	EC: 5.4.99.15	Maltose
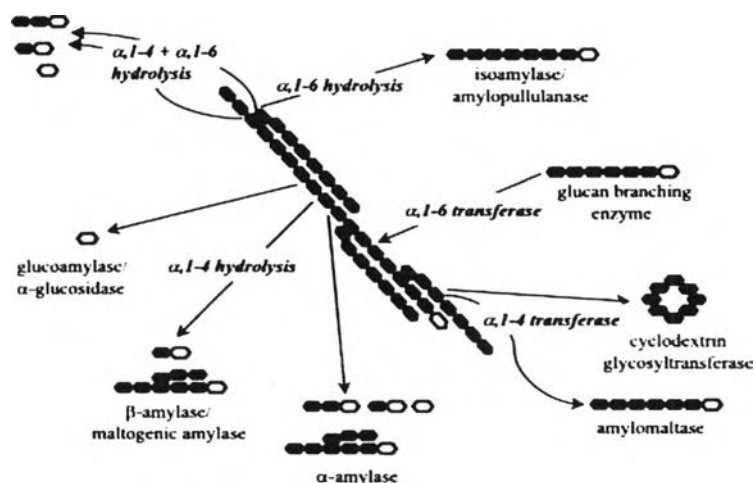
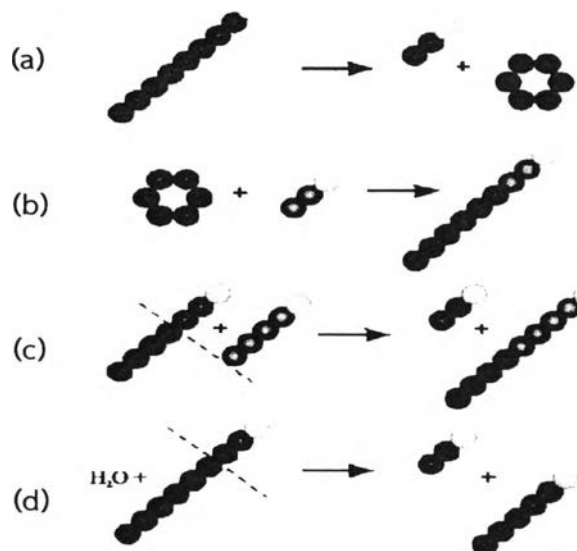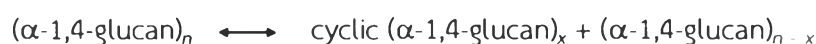Figure 1.2   The enzymes that catalyze the hydrolysis of glycosidic bond of starch (van der Maarel *et al.* 2002)

Figure 1.3   4-α-glucanotransferase (4αGTase) catalyzes four different reactions. (a) cyclization reaction, (b) coupling reaction, (c) disproportionation reaction and (d) hydrolysis reaction. The black circles are glucose residues; the white circles are reducing end of glucose residues (van der Veen *et al.* 2000)

$$(\alpha\text{-}1,4\text{-glucan})_m + (\alpha\text{-}1,4\text{-glucan})_n \longleftrightarrow (\alpha\text{-}1,4\text{-glucan})_{m-x} + (\alpha\text{-}1,4\text{-glucan})_{n+x}$$

The second is cyclization, an intramolecular transglucosylation. The reaction occurs within a single linear glucan to produce a cyclic glucan called cyclodextrins (CDs) as shown in this equation:

$$(\alpha\text{-}1,4\text{-glucan})_n \longleftrightarrow \text{cyclic } (\alpha\text{-}1,4\text{-glucan})_x + (\alpha\text{-}1,4\text{-glucan})_{n-x}$$

The third is coupling, a reverse reaction of cyclization. In this reaction, CDs rings are opened and act as donor, the enzyme transfers glucosyl units from CDs to short chain oligosaccharide producing longer chain oligosaccharide as product. Finally, hydrolysis reaction, the enzyme transfers glucosyl residues to water that acts as acceptor. Among these reactions, amylomaltase has high activity in disproportionation and cyclization reaction, but low activity in hydrolysis reaction (Takaha and Smith 1999). These four reactions can be catalyzed by both amylomaltase and CGTase, but there is a difference in the products of cyclization reaction. CGTase produces small-ring cyclodextrins (SR-CDs) while amylomaltase produces large-ring cyclodextrins (LR-CDs) as products. SR-CDs are cyclic oligosaccharides consist of 6, 7 and 8 glucose units connected by α-1,4 glycosidic linkage which is called α-, β-, γ-CDs, respectively (French 1957, Thoma and Stewart 1965). LR-CDs are cyclic oligosaccharides comprise of glucose residues linked by α-1,4 glycosidic bond with DP 9 onwards (Takaha and Smith 1999, Bhuiyan *et al.* 2003).

1.3 Amylomaltase

Amylomaltase (EC. 2.4.1.25) is an intracellular enzyme in 4-α-glucanotransferase group. It is found in microorganisms and involves in maltose metabolism (Boos and Shuman 1988) or synthesis/degradation of glycogen (von Mering *et al.* 2003). In plants, this enzyme is called disproportionating or D-enzyme which involves in starch metabolism (Colleoni *et al.* 1999, Critchley *et al.* 2001). Amylomaltase catalyzes the transfer of glucosyl residues from donor to glucosyl acceptor at free OH group of C4 through intermolecular and intramolecular transglucosylation to produce linear oligosaccharides and large-ring cyclodextrins (LR-CDs) with a degree of polymerization (DP) from 16 onwards, respectively (Takaha and Smith 1999, Endo *et al.* 2002). Amylomaltase was first found in *Escherichia coli* as maltose-inducible enzyme that involved in maltose metabolism (Doudoroff *et al.* 1949, Monod and Torriani 1950). Amylomaltase gene was reported in many bacterial strains such as *Streptococcus pneumonia* (Stassi *et al.* 1981), *Clostridium butyricum* NCIMB 7423 (Goda *et al.* 1997), thermophilic bacterium *Thermus aquaticus* (Terada *et al.* 1999), *Aquifex aeolicus* (Bhuiyan *et al.* 2003) and some archae such as hyperthermophilic *Thermococcus litoralis* (Jeon *et al.* 1997) and hyperthermophilic *Pyrobaculum aerophilum* IM2 (Kaper *et al.* 2005). D-enzyme was found in potato tuber (Peat *et al.* 1956), carrot roots (Manners and Rowe 1969), spinach leaves (Okita *et al.* 1979), Arabidopsis leaves (Lin and Preiss 1988), pea leaves (Kakefuda and Duke 1989). The three dimensional structure of amylomaltase from *T. aquaticus* (Przylas *et al.* 2000), *Thermotoga maritime* (Zhang *et al.* 2002), *Thermus thermophilus* (Barends 2007), *Thermus brockianus* (Jung *et al.* 2011), and *A. aeolicus* (Fujii *et al.* 2005) have been reported.

1.4 Applications of amylomaltase

Amylomaltase catalyzes the formation of LR-CDs through cyclization reaction. LR-CDs, a cyclic oligomer of glucose with DP from 16 onwards connected by α-1,4 glycosidic bond, are produced via cyclization reaction (Takaha and Smith 1999) catalyzed by amylomaltase (Srisimarat *et al.* 2011), D-enzyme (Takaha *et al.* 1988), glycogen debranching enzyme (Yanase *et al.* 2002), 4αGTase (Xu *et al.* 2014). LR-CDs with DP19-DP50 were produced by amylomalase from *C. glutamicum* (Srisimarat *et al.* 2011) while LR-CDs with DP17-DP100 were reported to be produced by D-enzyme from potato (Takaha *et al.* 1996). LR-CDs structure is a toroidal shape and a single helical V-amylose conformation (Figure 1.4) with hydrophobic inner cavity and hydrophilic surface (Gessler *et al.* 1999). Due to the hydrophilic surface, LR-CDs can dissolve in water and form inclusion complex with guest molecules such as fatty acid, alcohol (Endo *et al.* 2002), surfactant (Mun *et al.* 2009) and benzene (Harata 1981) by trapping guest molecules to hydrophobic cavity to improve solubility, stability and biological activity of guest molecules (Kim *et al.* 2011). LR-CDs are used in many applications: in food, pharmaceutical, cosmetic and paper industries (Takaha and Smith 1999). In pharmaceutical application, LR-CDs form inclusion complex with flurbiprofen and ibuprofen resulted in 14-fold and 2-fold in drug solubility, respectively (Baek *et al.* 2011, Baek *et al.* 2012). LR-CDs are also used as artificial chaperone for protein refolding (Machida *et al.* 2000).
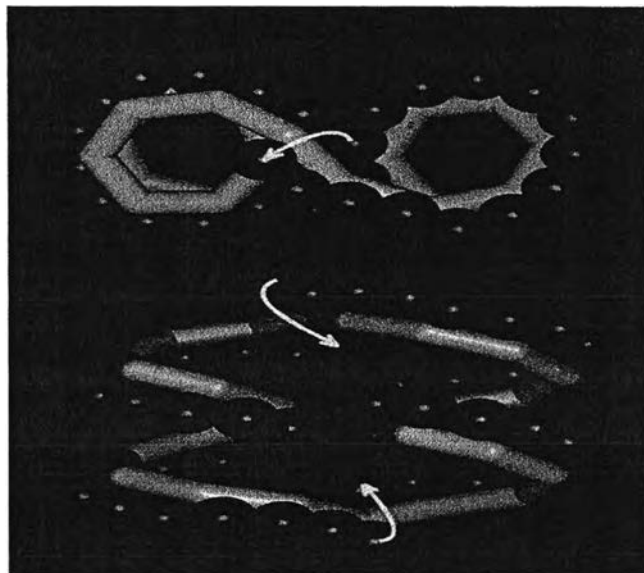
Figure 1.4   Top and side views of two left-handed single helices of LR-CDs with DP26. Oxygen atoms are represented in red, other atoms are shown in gray and band-flips are represented by yellow arrows (Gessler *et al.*, 1999).
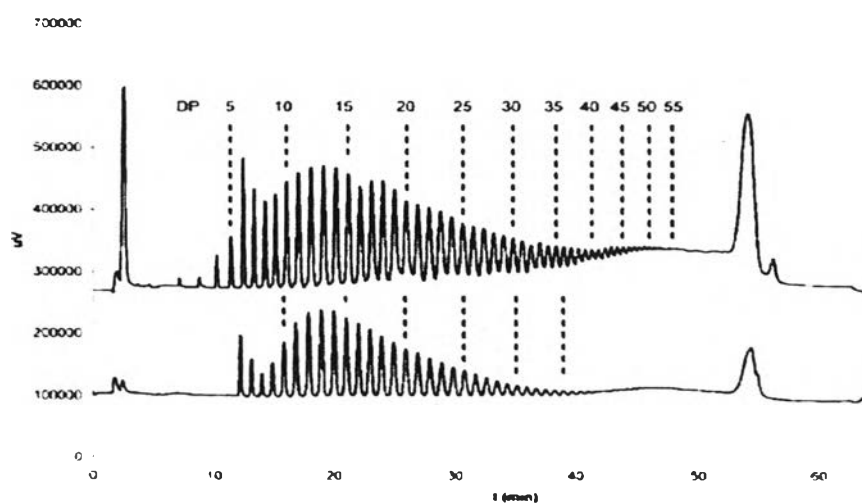

Figure 1.5   HPLC chromatogram of potato starch: starch modified with amylomaltase from *P. aerophilum* IM2 (upper) and native starch (lower) (Kaper *et al.*, 2005).

Amylomaltase is used to modify starch to produce thermoreversible gel. The enzyme hydrolyzes α-1,4 glycosidic linkage of amylose chain and transfers the glucosyl units to amylopectin chain, resulting in a change in side chain distribution (Figure 1.5) and thermoreversible property (Kaper *et al.* 2005, Hansen *et al.* 2008). Due to a change in side chain distribution, the melting temperature of modified starch is increasing when compared with native starch (Hansen *et al.* 2009). From previous reports, modified potato starch obtained from catalysis by amylomaltase from *T. thermophilus* HB8 (van der Maarel *et al.* 2005), and from *P. aerophilum* IM2 (Kaper *et al.* 2005) demonstrated thermoreversible property like gelatin. This modified starch is used in foods to replace gelatin from animal to make foods edible for vegetarian and muslim. Moreover, the modified starch is used to replace fat or cream in dairy products to improve creaminess in low-fat yogurt (Alting *et al.* 2009).

Amylomaltase is also used to produce glucoside products through intermolecular transglucosylation. Isomaltooligosaccharides (IMOs) with DP2-DP6 were synthesized by combination of amylomaltase and transglucosidase (Rudeekulthamrong *et al.* 2013). IMOs are non-digestible saccharides of glucose-based consisting of α-1,6 glucosidic linkage with/without α-1,4 glucosidic linkage. They act as prebiotic which activate growth of normal flora bacteria in digestion system of human, thus exerting health benefits. Furthermore, maltosylsucrose with anticariogenic property could be synthesized by amylomaltase from *C. glutamicum* using raw tapioca starch as donor (Saehu *et al.* 2013).

1.5 Acceptor specificity and synthesis of glucoside products

1.5.1 Synthesis of glucoside products by chemical method

Oligosaccharides and glucoside products are produced by linking of two compounds by forming a glycosidic bond. The glycosidic bond is formed by nucleophilic displacement (Figure 1.6) of leaving group (x) which binds to anomeric carbon of saccharides by an alkoxy group (ROH). In the presence of the promoter (a Lewis acid), the moving of leaving group is facilitated. The coupling of saccharides is based on donor reactivity, acceptor reactivity, promoter, substituents of saccharides and selectivity of the reaction to α- or β-anomer of the saccharides (Weijers et al. 2008). Besides acid activation (Fischer-Helferich method), the synthesis of oligosaccharides and glucoside products can be performed by Koenigs-Knorr method (glycosyl halide activation), acid activation, 1-O-alkylation (base activation) and Trichloroacetimidate (base-acid activation) (Schmitt et al. 1993) (Figure 1.7).

1.5.2 Synthesis of glucoside products by enzymatic method

1.5.2.1 Glycosidases

Glycosidases (EC 3.2.1.-) or glycosyl hydrolases catalyze the cleavage of glycosidic bonds. Glycosidases can be divided into 2 types, exo-glycosidases and endo-glycosidases (Trincone and Giordano 2006). Exo-glycosidases catalyze the transferring of glucosyl units from glucosyl donor (monosaccharides, oligosaccharides and glucosides) to non-reducing end of acceptor to produce glucoside products. The reaction catalyzed by endo-glucosidase gives a branched structure as products. The glycosyl-enzyme intermediate is produced in the reaction catalyzed by glycosidases, intereaction between this intermediate and water or glucosyl acceptor results in
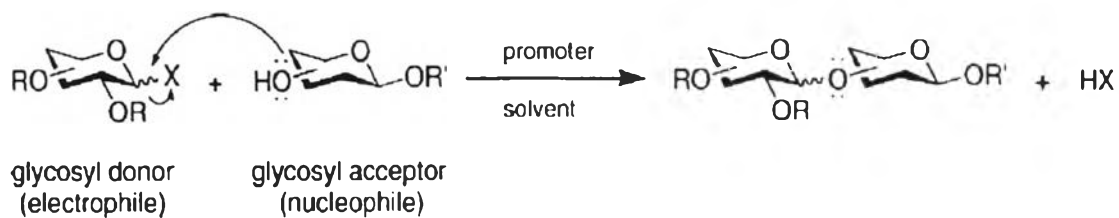
Figure 1.6   Formation of glycosidic bond by chemical synthesis (Weijers *et al.*, 2008).
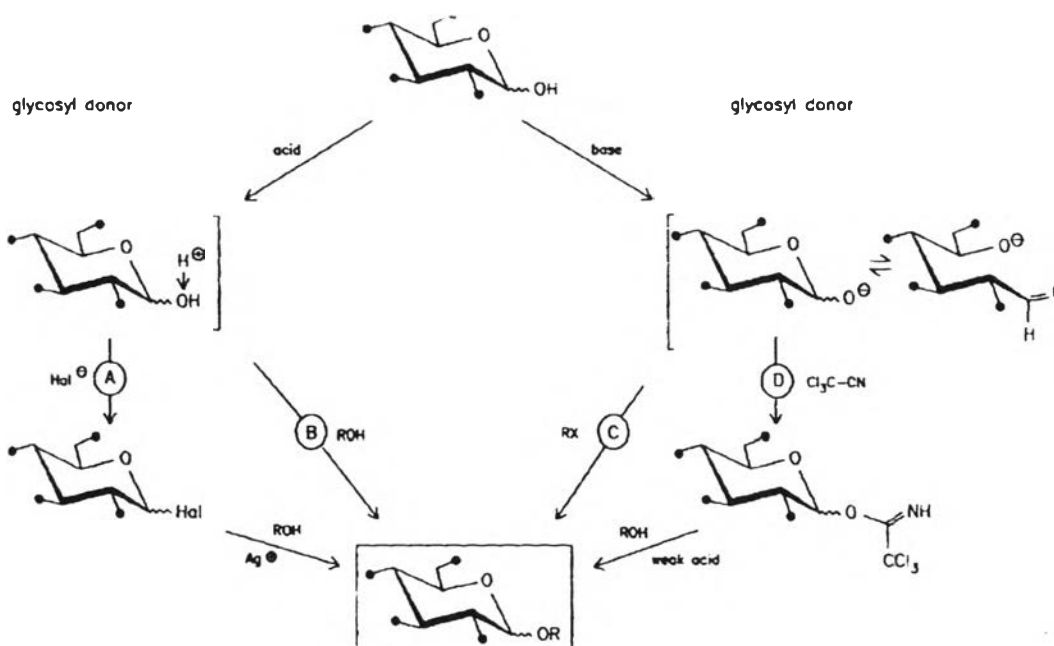

Figure 1.7   Schematic of the synthesis of oligosaccharides and glucoside products. A = Koenigs-Knorr method, B = Fischer-Helferich method, C = 1-*O*-alkylation and D = Trichloroacetimidate (Schmidt, 1986).

hydrolyzed products or glucoside products, respectively (Figure 1.8). The advantages of glycosidase are simple reaction and tolerance to many organic solvents while the disadvantage is the low product yield (Palcic 1999).

1.5.2.2 Glycosyltransferases

Glycosyltransferases (EC 2.4.x.y) catalyze the transferring of sugar moieties from donor to acceptor and glycosidic linkage is formed. The acceptors of these enzymes are monosaccharides, oligosaccharides, protein, nucleic acid, lipid or other small molecules with OH group such as flavonoids and alcohols (Trincone and Giordano 2006, Schuman *et al.* 2007). Glycosyltransferases are classified into 2 groups: Leloir pathway enzymes and non-Leloir pathway enzymes (Figure 1.9). The donors of Leloir pathway enzymes are carbohydrate or a nucleotide sugar (CMP, UDP, GDP, TDP) while the donors of non-Leloir pathway enzymes are disaccharide, oligosaccharide or sugar monophosphate by phosphorylation. Then the enzymes transfer the glucosyl units from donor to acceptor to produce glucoside products. There are several enzymes in glycosyltransferase family such as phosphorylase, CGTase and amylomaltase. The reaction catalyzed by glycosyltransferases gave high product yield, but these enzymes are not stable in organic solvents (Wong *et al.* 1982).

1.5.2.3 Previous studies on enzyme synthesis of glucoside products

The synthesis of alkyl glucosides, flavonoid glucosides, oligosaccharides and glucosides by enzymatic method have been reported as followings:
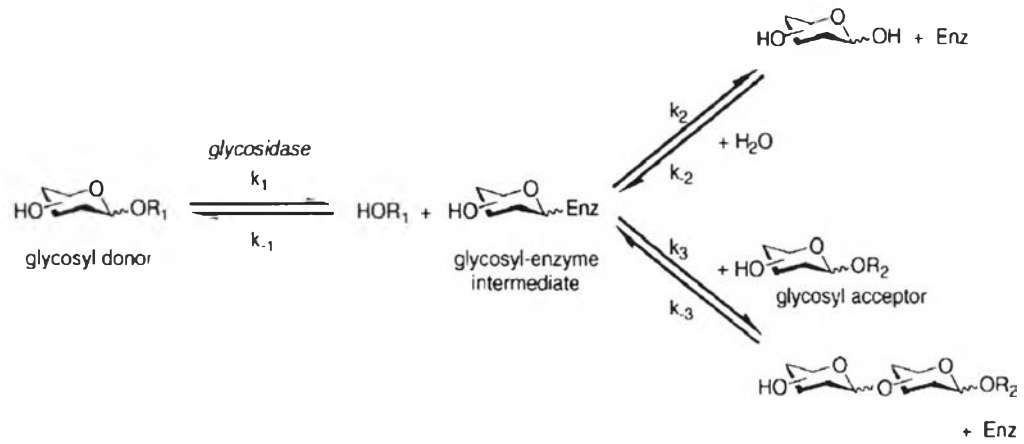
Figure 1.8   The synthesis of glucoside products or oligosaccharides by glycosidases (Weijers *et al.*, 2008).
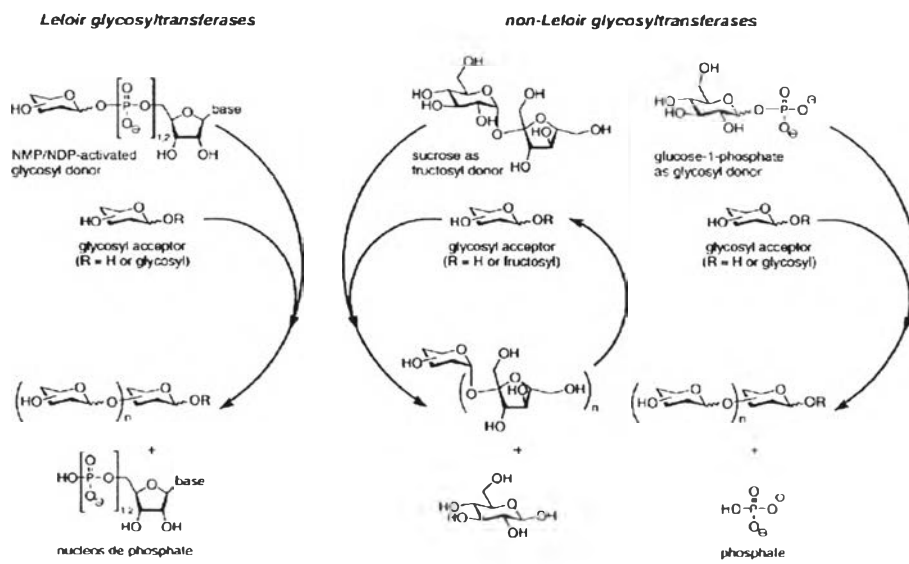

Figure 1.9   Synthesis of glucoside products by Leloir or non-Leloir glycosyl-transferases (Weijers *et al.*, 2008).

Synthesis by 4αGTase : by CGTase

In 1992, Kitahata and colleagues reported acceptor specificity and synthesis of galactosyl-glucoside by CGTase from *Bacillus stearothermophilus*. They found that this enzyme was specific to glucose structure with free OH group at C1-C4. The glucoside products were synthesized by CGTase using soluble starch as donor and galactose as acceptor resulted in 4 main products with D-glucosyl O-β-D-galactosyl-(1,4)-β-D-glucoside as the smallest product.

- In 1995, Terada and colleagues reported the production of hesperidin monoglucoside and hesperidin diglucosides by CGTase from an alkalophillic *Bacillus* sp. These glucosides could prevent the formation of crystal of hesperidin that caused turbidity in canned orange.

- In 1996, Kometani and colleagues reported acceptor specificity of CGTase from an alkalophilic *Bacillus* species and the synthesis of glucosyl rhamnose. The enzyme showed specificity to glucose, xylose, arabinose, fucose, fructose, mannose, galactose and rhamnose with free OH group at C2-C4. The glucoside products were synthesized by CGTase which transferred glucosyl units from soluble starch donor to rhamnose acceptor to produce glucopyranosyl-α-1,4-rhamnopyrnose as product.

- In 1998, Park and colleagues reported characteristics of transglycosylation reaction of CGTase from *Bacillus macerans* using starch as a glucosyl donor and various saccharide acceptors. This enzyme preferred saccharide with OH configuration at C2-C4 as same as glucose such as D-xylose and L-sorbose.

- In 2000, Tachibana and colleagues reported that 4αGTase from *Pyrococcus kodakaraensis* KOD 1 preferred saccharide acceptors with pyranose structure of which the OH configuration at C2-C4 is similar to glucose.

- In 2001, Martin and colleagues reported the synthesis of maltooligosaccharides via the acceptor reaction catalyzed by CGTase from *Thermoanaerobacter* sp. Maltose was the best acceptor, however, the enzyme activity was decreased when the length of acceptor was increased. Then the homologous series of maltooligosaccharides (G_n) with 63-79% yield were synthesized by using soluble starch as donor and glucose as acceptor, the DP of maltooligosaccharides depended on the ratio of starch:glucose.

- In 2007, Prousoontorn and Pantatan reported the production of *2-O-α-glucopyranosyl* L-ascorbic using immobilized CGTase from *Paenibacillus* sp. A11. Four types of supports were tested to immobilize the enzyme: alumina, silica, carbon and chitosan. Alumina was a suitable support with high immobilized activity. Then *2-O-α-glucopyranosyl* L-ascorbic with 2.92% recovery yield was produced by immobilized CGTase using β-CD as donor. The activity of immobilized CGTase was decreased to about 26% of initial activity after using for 3 cycles.

- In 2010, Wongsangwattana and colleagues reported the synthesis of cellobiose-containing oligosaccharides by CGTase from *Paenibacillus* sp. A11. Firstly, various saccharides were tested to determine the acceptor efficiency in transglucosylation reaction using β-CD as donor. For

monosaccharide acceptors, this enzyme preferred pyranose ring with free OH at C2-C4, and sorbose and glucose were efficient acceptors in this group with 57% and 54% yield. For disaccharide acceptors, this enzyme preferred β-(1,4) linkage and cellobiose was efficient acceptor, the yield obtained were 78%. Cellobiose was selected for glucosides synthesis, the products were glucose-α-(1,4)-cellobiose and glucose-α-(1,4)-glucose-α-(1,4)-cellobiose which could not be cleaved by α-amylase.

- In 2012, Mathew and colleagues reported synthesis of piceid glycoside by CGTase from. The various donors were studied: glucose, maltose, sucrose maltotriose and α-CD. The results showed that α-CD was the most efficient donor followed by maltodextrin. The yield of piceid glucoside was 72.1%.

- In 2012, Mathew and Adlercreutz reported the synthesis of dodecylglucooligosides by CGTase using α-CD or starch as donor and dodecyl -D-glucoside ($C_{12}G_1$) or dodecyl β-D-maltoside ($C_{12}G_2$) as acceptor which produced dodecylglucoheptaoside and dodecyl maltooctaoside, respectively. α-CD was an efficient acceptor in coupling reaction while starch was an efficient acceptor in transglucosylation reaction and the yield of products from using of α-CD acceptor was higher than using of starch acceptor.

Synthesis by 4αGTase : by amylomaltase

- In 2013, Saehu and colleagues reported the synthesis of maltooligosylsucrose by amylomaltase using sucrose and raw tapioca starch as glucosyl acceptor and donor, respectively. The main products

were maltosyl-, maltotriosyl-, maltotretraosyl- and maltopentaosyl-fructosides with 82% yield. These products showed anticariogenic property by inhibition of GTase activity of the oral bacteria *Streptococcus mutans*.

- In 2013, Rudeekulthamrong and colleagues reported the synthesis of IMOs by using the combination of amylomaltase and transglucosidase with maltotriose substrate. The products obtained were isomaltose, isomatotriose and isomaltotetraose. These short chain IMOs connected by α-1,6 and α-1.4 linkages.

Synthesis by other hydrolases and transferases

- In 1994, Nishimura and colleagues reported acceptor specificity in the glucosylation reaction of *B. subtilis* X-23 α-Amylase towards various phenolic compound. They found that hydroquinone, resorcinol, catechol, catechins, and kojic acid were efficient acceptor for α-amylase.

- In 1999, Lee and colleagues reported on transglycosylation of naringin by *B. stearothermophilus* maltogenic amylase to give mono-, di- and triglycosylnaringins. The enzyme transferred maltose to glucose units of naringin connected by α-1,6 linkage. These glucosides showed 250 times higher solubility in water, but 10 times less bitter than the parent naringin.

- In, 1999, Meulenbeld and colleagues reported the synthesis of catechin glucoside by glycosyltransferase D from *S. mutans* GS-5. Two types of catechin glucosides: (+)-catechin-4'-*O*-a-D-glucopyranoside and (+)-catechin-4', 7-*O*-a-di-D-glucopyranoside were produced with 90% recovery using sucrose as acceptor.

- In 2000, Kim and and colleagues reported the synthesis of alkyl fructoside from sucrose donor and methanol acceptor catalyzed by levansucrase from *Rahnella aquatilis*. The effect of methanol concentration, sucrose concentration and temperature were determined. They found that this enzyme was stable in 20-80% methanol, and the highest yield of glucoside products was found in the reaction containing 40% methanol. In addition, this enzyme was active at low temperature (10 °C). The glucoside product synthezied was methyl β-D-fructoside.

- In 2001, Chaen and colleagues reported the production of novel oligosaccharides from L-sorbose, maltose, or sucrose acceptor and β-D-glucose-l-phosphate donor using Kojibiose Phosphorylase. Three novel oligosaccharides were detected: α-D-glucopyranosyl-(1,5)-α-L-sorbopyranose, α-D-glucopyranosyl-(1,2)-α-D-glucopyranosyl-(1,4)-D-glucopyranose and α-D-glucopyranosyl-(1,2)-β-D-glucopyranosyl-(1,2)-β-D-fructofuranoside. The sorbose glucoside could be hydrolyzed by α-glucosidase which produced glucose and sorbose while both maltose and sucrose glucoside could not be hydrolyzed by α-glucosidases, glucoamylase and CGTase.

- In 2005, Watanabe and colleagues reported the enzymatic synthesis of cyclic tetrasaccharide glucosides by kojibiose phosphorylase from *T. brockii* ATCC 35047. The enzyme was incubated with trehalose donor and a cyclic tetrasaccharide acceptor at 55 °C for 72 hours and four types of glucosides were produced. This enzyme transferred the glucosyl units to OH group at C2 of cyclic saccharide, the main product was 2-*O*-α-D-

glucopyranosyl cyclic tetrasaccharide. It was found that the solubility of 2-O-α-D-glucopyranosyl cyclic tetrasaccharide was lower than cyclic saccharide.

- In 2006, Moon and colleagues reported the synthesis and characterization of epigallocatechin gallate glycoside catalyzed by glucansucrase from *Leuconostoc mesenteroides* B-1299CB using sucrose as acceptor. Three types of glucosides were observed: epigallocatechin gallate 7-*O*-α-D-glucopyranoside, epigallocatechin gallate 4'-*O*-α-D-glucopyranoside and epigallocatechin gallate 7, 4'-*O*-α-D-glucopyranoside. All glucosides showed higher browning resistant activity and water solubility than epigallocatechin gallate.

- In 2009, Svensson reported synthesis of alkyl glycosides by α-amylase from *Aspergillus oryzae* using starch as glycosyl donor and methanol, ethanol, propanol, butanol and pentanol as acceptors. The alkyl glucosides were not observed when hexanol or octanol were used as acceptor. Moreover, hexyl glucoside and octyl glucoside were also synthesized by a novel thermostable β-glucosidase from *Thermotoga neapolitana* using p-nitrophenyl-β-glucopyranoside as donor and hexanol or octanol as acceptor

- In 2009, Kim and colleagues reported synthesis of alkyl glucosides by *L. mesenteroides* dextransucrase using various types of alcohols (methanol, ethanol, 1-propanol, 1-butanol, 2-propanol, tert-butanol, or 3-methyl-1-butanol) and sucrose as glucosyl acceptor and donor, respectively. The glucosides were analyzed by TLC and HPLC, the yield of

glucosides from primary alcohol acceptor (methanol, ethanol, 1-propanol, 1-butanol or 3-methyl-1-propanol) were 38%, 40%, 38%, 50%, or 45% (mol/mol), respectively whereas the yield from secondary alcohol (2-propanol) was 24%. However, the glucosides from tert-butanol acceptor could not be detected. Then methyl α-D-glucoside and ethyl α-D-glucoside were synthesized using methanol and ethanol as acceptor. These alkyl glucosides had twice less emulsification activity than Triton X-100 surfactant.

- In 2010, Kurakake and colleagues reported acceptor specificity of β-xylosidase from *A. awamori* K4. The enzyme preferred sorbitol, mannitol to other saccharides and gave 6-*O*-β-xylosyl-sorbitol and 1(6)-*O*-β-xylosyl-mannitol with 77.3% and 73.7% recovery yield, respectively.

Synthesis of palatinose glucosides by phosphorylase

- In 2007, Takahashi and colleagues reported the synthesis of palatinose glucosides by phosphorylase from *T. brockii* using β-D-glucose-1-phosphate as donor. Two types of glucoside products were found: trisaccharide (2^G-α-D-glucopyranosyl-palatinose) and tetrasaccharide (2^G(2-α-D-glucopyranosyl)$_2$-palatinose).

1.6 Objectives

From previous studies, amylomaltase from *C. glutamicum* was successfully cloned and expressed in *E. coli*. Enzyme characterization and the production of LR-CDs were characterized. This enzyme produced LR-CDs with DP19-DP50, main products were DP27-DP29 (Srisimarat *et al.* 2011). Then this enzyme was used to

synthesize maltooligosylsucrose with an anticariogenic property (Saehu *et al.* 2013). In the present study, we here focus on determination of acceptor specificity of this amylomaltase and the synthesis of glucoside products from the most suitable acceptor together with characterization of the glucoside products.

Research steps:

1. Preparation and purification of amylomaltase from *C. glutamicum*

2. Determination of acceptor specificity and synthesis of glucoside products

3. Optimization of the synthesis of glucoside products

4. Large scale preparation, product separation and identification

5. Characterization of glucoside products