Chapter 4
Synthesis of Polysaccharides III: Sucrase as Catalyst

Satoshi Kimura and Tadahisa Iwata

Abstract Sucrase-type glycosyltransferases that classified into non-Leloir glycosyltransferases, named glucansucrase and fructansucrase, catalyze in transfer of either a glucose or a fructose from sucrose to produce glucans or fructans. The reactions need only a renewable carbon resource, such as sucrose, and proceed very efficiently, with high yields, with regio- and stereoselectivity, and in one-pot water-based system. This chapter provides an overview of the glucansucrase and fructansucrase enzymes, their reaction, and product specificity. Finally, we discuss the potential applications of α-glucans produced by glucansucrase in new bio-based materials.

Keywords α-Glucan · Fructan · Glucansucrase · Fructansucrase · Glucosyltransferase · Sucrose

4.1 Introduction

Polysaccharides, natural polymers composed of sugar units linked via glycosidic bonds, have been considered as interesting bio-based materials for utilization in many applications such as plastics and biomedical field with currently increasing amount of researches. The advantages are that they are made from renewable resources supporting the trend to reduce the consumption of plastics made from petrochemicals, and with the concept of carbon neutrality, they can be regarded as eco-friendly materials [1]. Plants typically produce polysaccharides such as well-known cellulose and hemicelluloses such as xyloglucan, xylan, and glucomannan. However, hemicelluloses are branched, and they are extracted from wood via alkali or acid process leading to chain degradation that lowers the molecular weight. Besides, microorganisms can synthesize many polysaccharides in the culture

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medium as well such as pullulan from *Aureobasidium pullulans*; curdlan from *Agrobacterium*, *Rhizobium*, and *Cellulomonas*; dextran from *Leuconostoc* and *Streptococcus*; and hyaluronic acid from *Streptococcus* and *Pasteurella* [2]. In any case, however, it is required complicated purification step in order to purify the target polysaccharides, and the limitation of the direct production of polysaccharides by microorganisms is the difficulty in structure and composition control due to the nature of each producer.

In vitro enzymatic polymerization of polysaccharides is convenient and environmentally friendly method for production of polysaccharides. Sucrase-type glycosyltransferases classified into non-Leloir glycosyltransferases have been employed as catalysts for the practical synthesis of polysaccharides by both polymerization and modification. It is to be mentioned that sucrases, e.g., glucansucrase and fructansucrase, belong to glycosidases (EC 3.2.1). These enzymes catalyze in transfer of either a glucose or a fructose moiety of sucrose to produce glucans or fructans of different types with respect to glycosidic linkages and side chains. The reactions proceed very efficiently, with high yields, with regio- and stereoselectivity, and in one-pot water-based system. The background of this convenient synthetic pathway is the high energy of the glycosidic bond of sucrose, which is similar to that of nucleotide-activated sugars. The simplified reaction manners are represented as follows.

Glucansucrase: n Sucrose → Glucan + n Fructose
Fructansucrase: n Sucrose → Fructan + n Glucose

In this chapter, biochemical characterizations of glucansucrases and fructansucrases are summarized, and their recent applications with a focus on in vitro-synthesized α-glucan by glucansucrase are described.

4.2 Glucansucrase

Glucansucrases are extracellular enzymes mainly produced by lactic bacteria *Lactococcus*, *Leuconostoc*, and oral *Streptococcus* [3]. Glucansucrases catalyze the synthesis of high molecular weight D-glucose polymers named glucans from sucrose (Fig. 4.1). Dextran, α(1 → 6)-glucan is the first reported and most common glucan synthesized by a kind of glucansucrase, was one of the first biopolymer to be produced on an industrial scale in 1948 [4]. The glucansucrase responsible for the synthesis of dextran was first reported in *Leuconostoc* [5] and was named as dextransucrase (EC 2.4.1.5). Amylosucrase (EC 2.4.1.4) is the most extensively studied glucansucrase [6–8]. Amylosucrase was found in the genus *Neisseria* and named because of its enzymatic conversion of sucrose to a glycogen- or amylopectin-like polymer [9]. Until now, amylosucrase gene from *Neisseria polysaccharea* was cloned and heterologously expressed in *Escherichia coli* [10], followed by reports of its three-dimensional structure [11, 12].

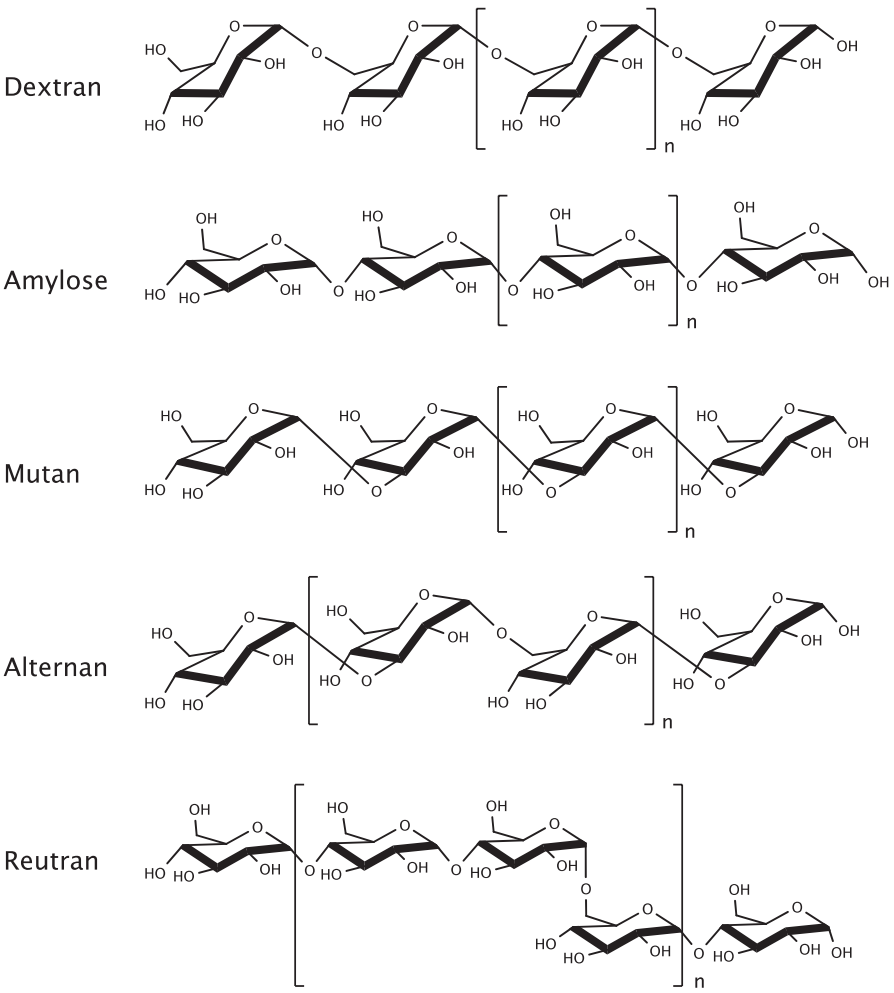

Fig. 4.1 The basic structures of the α-glucans synthesized by glucansucrases. The α-glucans are classified according the dominant linkage type in the main chain

Glucansucrases of oral streptococci, like *Streptococcus mutans*, play a key role in the cariogenesis process, as the synthesized glucans enhance the attachment and colonization of cariogenic bacteria [13, 14]. Therefore, in order to develop vaccines against dental caries, studies for the isolation of the gene encoding for these glucansucrases of oral streptococci were initiated more than 40 years ago. The first genes encoded glucansucrases (named *gtf*) were cloned from *Streptococcus downei*, using γ phage as cloning vector and screening on sucrose-containing medium [15]. The term GTF (glucosyltransferases) has been the one preferred by researchers with oral bacteria; this term does carry to confusion with the nucleotide sugar-dependent intercellular glycosyltransferases [16]. In the sucrase-type enzymes, energy

necessary to catalyze all the reactions comes only from the cleavage of the glycosidic bond of sucrose. The mediation of nucleotide-activated sugars or cofactors is not necessary. The term glucansucrase has been favorably used in recent years, the specificity of the enzyme being indicated by a name derived from the main glucan produced, e.g., α(1 → 6)-glucan (dextransucrase) and α(1 → 4)-glucan (amylosucrase).

A long-standing question about glucansucrase relates to the mechanisms of chain elongation and the determinants of type of glycosidic linkage introduced to the growing glucan chain. As nucleotide sequences of glucansucrases became available, multiple alignments of the deduced amino acid sequences revealed conserved unique features which may explain which amino acid residues or domains are responsible for these properties. Of the oral streptococci, *S. mutans* produces three distinct glucansucrases, while in *S. sobrinus*, the closely related *S. downei* and *S. salivarius* each produce four glucansucrase genes (Table 4.1) [17]. In contrast, *S. gordonii*, *S. sanguinis*, and *S. oralis* make only a single glucansucrase.

4.2.1 Catalytic Mechanism of Glucansucrase

The sequences of about 500 different glucansucrase genes including ORF fragment are listed on the CAZY database of Carbohydrate Active Enzymes (http://www.cazy.org/) [18–20]. And more than 60 glucansucrases have been biochemically characterized. In the CAZY classification system, all glucansucrases except amylosucrases are classified as family GH70. Amylosucrase is the only enzyme of family GH13 displaying polymerase activity and is clearly unique in the family GH13 that mainly contains starch-degrading enzymes. These enzymes from families GH13 and GH70 are also known as the part of the α-amylase superfamily and are classified in clan GHH [18–20]. In the α-amylase enzymes, the central catalytic core of these enzymes is predicted to have alternating α-helixes and β-sheets, in an arrangement the same as found in the (β/α)8 barrel found in amylase. Although these enzymes catalyze transglycosylation or hydrolysis reactions on differently linked α-glucan polymers, they use the same set of key amino acid residues to catalyze their reaction [21–24]. During the 2000s, the role of these residues and the mechanism of the reaction have been extensively studied in GH13 enzymes such as

Table 4.1 Glucansucrases in *Streptococcus salivarius* (ATCC 25975)

Enzyme	Mw (10^{-3} x M_r)	Glucan (water solubility)	
GtfJ	168	100% α(1→3)-	(insoluble)
GtfK	176	100% α(1→6)-	(soluble)
GtfL	157	50% α(1→6)- / 50% α(1→3)-	(insoluble)
GtfM	171	95% α(1→6)-	(soluble)

Neisseria polysaccharea amylosucrase [25], *Aspergillus oryzae* α-amylase [26], and *Bacillus circulans* cyclodextrin glucanotransferase [27]. A decade later, final evidence for the catalytic mechanism of GH70 glucansucrase came from the three-dimensional structure and its complexes with sucrose or maltose [23].

General reaction mechanism for glucansucrase is shown in Fig. 4.2. Based on the sequence homology within the α-amylase superfamily, the corresponding catalytic residues in glucansucrases had been identified, and the mechanism was proposed to be similar [24]. The most important catalytic residues are a nucleophile (aspartate), a general acid/base (glutamate), and a transition-state stabilizer (aspartate). This mechanism is based on a detailed structural analysis of *B. circulans* 251 CGTase in complex with intact substrate and on a covalent intermediate of the same enzyme [27]. In this mechanism, first the glycosidic linkage of the sucrose is cleaved, resulting in a covalent α-glucosyl-enzyme intermediate; in the second half-reaction, the glucosyl moiety is transferred to an acceptor with retention of the α-anomeric configuration. After formation of the covalent intermediate, fructose is released, and the glucosyl moiety is transferred to an accepting sugar (transglycosylation) or to a water molecule (hydrolysis). The excellent review articles have been also published to provide detailed information of structure and function of glucansucrase [28–30].

4.2.2 Synthesis of α-Glucans by Glucansucrases

Different kinds of α-glucans with different sizes and structures, depending on the glucansucrase-producing bacterium, are synthesized. Based on their main glycosidic linkage type, these α-glucans are divided into five categories: dextran, amylose, mutan, alternan, and reuteran (Fig. 4.1). This structural variability results in a wide range of physicochemical properties, which may be suitable for different applications.

Dextran Dextran is a water-soluble α-glucan mainly composed of α(1 → 6) linkage connected by varying amounts and arrangements of α(1 → 2), α(1 → 3), and

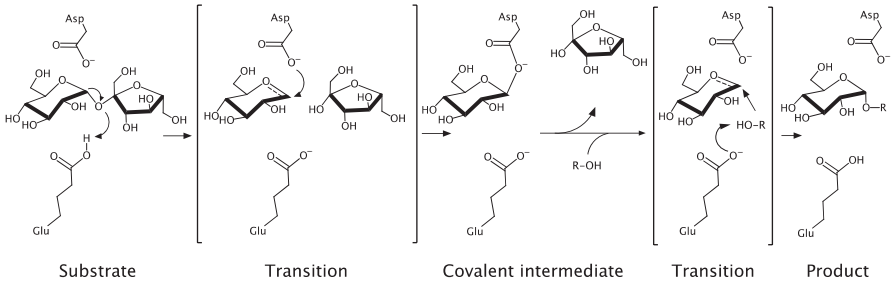

Fig. 4.2 General reaction mechanism of glucansucrase catalyst to produce glucan from sucrose with liberation of fructose

Table 4.2 Examples of dextran synthesized by glucansucrases from sucrose

Enzyme	Linkage compositon (%)				Ref.
	α(1→2)	α(1→3)	α(1→4)	α(1→6)	
Leuconstoc mesenteroides NRRL B-512F DSRS		5		95	[40]
Leuconstoc citreum B-1299 DSRE	5	10	3	81	[72]
Leuconstoc citreum B-1299 BSR-A	37			63	[73]
Leuconstoc citreum BSR-B		50		50	[74]
Weissella cibaria DSRWC				100	[39]
Lactobacillus reuteri 180 Gtf180		31		69	[47]
Streotococcus mutanss GS5 GtfD		30		70	[37]
Streotococcus salivarius GtfJ				100	[12]

α(1 → 4) linkages (Fig. 4.1, Table 4.2). The glucansucrase responsible for the synthesis of dextran is designated as dextransucrase and was first reported in *Leuconostoc* [5]. Subsequently, dextransucrases from various species of the genera *Lactobacillus*, *Streptococcus*, and *Weissella* were also identified and characterized [31–33]. The dextran is widely used in separation technology, biotechnology, and several applications in medicine [34, 35]. Dextran for clinical and technical applications is marketed in most developed countries all over the world. Of industrial relevance is the dextran produced by *Leuconostoc mesenteroides* NRR B- 512F DSR-S; the dextransucrase of this strain converts sucrose into a high molar mass (up to 1 MDa) polymer with 95% α(1 → 6) linkages in the main chains and 5% α(1 → 3)-branching linkages [36–38]. This native or partially degraded dextran and its derivatives have found many industrial applications in medicine (e.g., blood plasma expander, anticoagulant, and antithrombotic agents), pharmacy (e.g., lubricant and carrier), food (e.g., thickening, stabilizing, and gelling agent), and biotechnology (e.g., chromatography matrix) [38–40].

Another intensively studied dextransucrase is the *Lactobacillus reuteri* 180 Gtf180 GS producing an α-glucan with a high molecular weight of 30 MDa containing 69% of α(1 → 6) linkages plus single α(1 → 3) linkages in linear (21%) and branched (13%) orientations [41]. Notably, different α-glucans with unique highly branched structures have been reported in *Leuconostoc* strains. The *Leuconostoc citreum* NRRL B-1299 was found to synthesize a dextran polymer with mostly α(1 → 6) linkages but also containing about 30% of α(1 → 2) linkages, as well as a limited amount of α(1 → 3)-branching linkages [42–44]. This strain encodes six different glucansucrases, namely, DSR-A, DSR-B, DSR-E, DSR-M, DSR-P, and BRS-A [43].

Amylose Amylosucrase can catalyze three types of enzymatic reactions when sucrose is the only substrate: polymerization, isomerization, and hydrolysis [45].

The polymerization reaction, a unique characteristic of amylosucrase, synthesizes α-glucan with only α(1 → 4) linkages and has no need for any primer. Recombinant amylosucrase from *Neisseria polysaccharea* synthesizes amylose with DP of 35–58 in vitro. By changing only the initial sucrose concentration, it was possible to obtain amyloses with different morphology and structure [46]. Simultaneously, amylosucrase produces a certain number of sucrose isomers, turanose and trehalulose, through isomerization reactions, and catalyzes a hydrolysis reaction releasing glucose and fructose from sucrose [45]. In addition, in the presence of sucrose and extra glycosyl acceptors, amylosucrase has transglycosylation capacity to attach glucose molecules from sucrose to glycosyl acceptors, such as glycogen [47, 48], starch [49], and flavanone [50]. These unique reactions make it a vital transglucosylation tool in producing novel polysaccharides and carbohydrate-based bioactive compounds.

Using the self-assembly process of α(1 → 4)-glucans produced by amylosucrase, several amylose microbeads and their applications were reported. Amylose-single-walled carbon nanotube microbeads are the first exploration of enzymatic synthesis of microparticles by amylosucrase [51]. Amylose magnetic microbeads were also prepared by the similar enzymatic approach in the presence of iron oxide nanoparticles [52]. The produced microbeads had a well-defined spherical shape and exhibited excellent magnetic responses and dispersibility in aqueous solutions.

Mutan Mutan polymers are water-insoluble α-glucan mainly composed of α(1 → 3) linkage in the linear backbone and minor amounts of α(1 → 6) linkages. Mutansucrase-synthesizing mutan polymers are mainly found in *Streptococcus* strains. The ability of *Streptococcus mutans* and *Streptococcus sobrinus* to convert sucrose into water-insoluble glucans was found to be important in the etiology of dental caries by facilitating the colonization of tooth surfaces [53]. Consequently, glucansucrases are regarded as potential targets for anticaries drugs [54]. Mutansucrases have also been found in *Lactobacillus* and *Leuconostoc* strains [55, 56]. Mutan is regarded as a potentially low-cost polymer, which may be used to develop new bio-based materials [57, 58]. In particular, chemical modification of mutans to ester derivatives has shown to improve the thermoplasticity of this polysaccharide (see below for further details) [57, 58]. Moreover, chemically sulfated mutan showed fibrinolytic, anti-inflammatory, and antimicrobial properties [59–62]. The use of mutan for a variety of applications as fibers, films, and resins has been patented. The in vitro-synthesized mutan by recombinant mutansucrase (GtfJ from *S. salivarius* ATCC 25975) showed only α(1 → 3) linkage without any branching structure, which is difficult to obtain from native sources.

A transmission electron micrograph of in vitro-synthesized mutan is shown in Fig. 4.3 (Kimura and Iwata unpublished data). The synthetic mutan are wavy fibril-like crystals, which aggregated into plates. By electron diffraction analysis, it was indicated that the glucan chain was not extended parallel to the fibril axis as with other fibrillar polysaccharides, such as cellulose and chitin, but folded in the fibrils

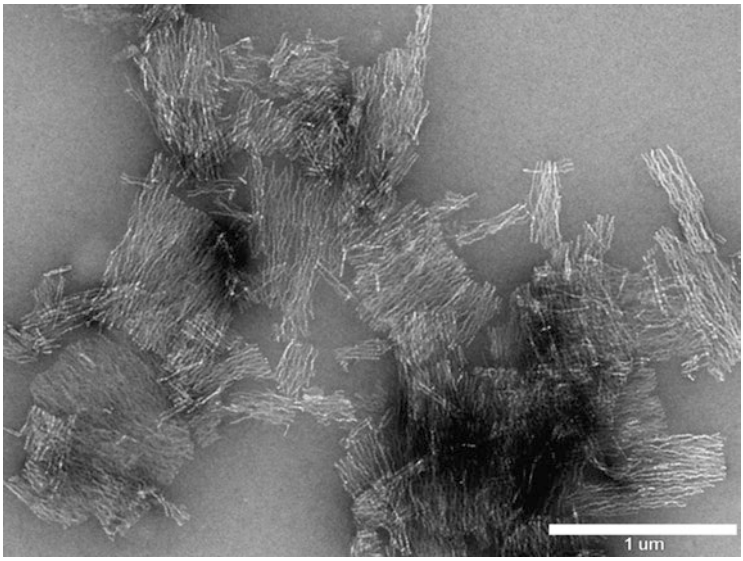

Fig. 4.3 Transmission electron micrograph of in vitro-synthesized mutan. (Kimura and Iwata unpublished data)

[63]. Several structural studies of mutan-like glucans have been reported by X-ray diffraction analysis [64–67]. In either case, however, regenerated mutan derived from the precipitate of an alkaline extraction from fungi cell walls was used, and the detailed structures of mutan have not been elucidated. The in vitro-synthesized mutan was a pure and completely linear α(1 → 3)-glucan without branches; for the first time, it was confirmed the morphology of native mutan [63].

Alternan Alternan, with alternating α(1 → 6) and α(1 → 3) linkages, was found in *L. mesenteroides* NRRL B-1355 [68, 69]. Due to its backbone structure of alternating α(1 → 6) and α(1 → 3) linkages, alternan is resistant to enzymatic digestion by most known mammalian and microbial hydrolytic enzymes [70]. These properties make alternan valuable as low-calorie food additive [71]. So far the only enzymes reported to hydrolyze alternan are isomaltodextranases and alternanase [70–73].

Reuteran Reuteran is a water-soluble branched α-glucan mainly composed of α(1 → 4)-glucan segments interconnected by single α(1 → 6) linkage. Only two reuteransucrase have been characterized, both of them present in *Lactobacillus reuteri* strains and producing reuteran polymers differing in the amount of α(1 → 4) and α(1 → 6) linkages [74, 75]. Reuteran has been described as a potentially health-promoting food ingredient.

4.3 Fructansucrase

Fructansucrases transfer the fructose units of sucrose onto polysaccharides or appropriate acceptors with release of glucose. Fructans, thus produced, are either levan composed of β(2 → 6)-linked fructose residues by levansucrase or inulin composed of β(2 → 1)-linked fructose residues by inulosucrase. Figure 4.4 has shown the typical structure of levan and inulin. When sucrose is used as the acceptor in the initial priming reaction, synthesized fructans contain a nonreducing glucose unit at the end of the chain. In bacteria, fructansucrases are extracellular enzymes; levansucrases are widely distributed in both gram-positive and gram-negative bacteria, while inulosucrases are exclusively present in lactic acid bacteria. Most of the research of fructansucrases has been performed on levansucrases, in particular on enzymes from *Bacillus* spp. [76–79] and *Zymomonas* spp. [80–83].

Fructansucrases cleave the glycosidic bond of sucrose and use the released energy to couple a fructose unit (i) to a growing fructan chain (transfructosylation), (ii) to sucrose, (iii) to water (hydrolysis), or (iv) to another acceptor (such as raffinose) [84, 85]. Because sucrose is used as the acceptor in the initial priming reaction, bacterial fructans contain a nonreducing glucose unit at the end of the chain [86] (Fig. 4.4). In the initial reaction of fructansucrases, the fructose of a sucrose molecule is coupled by the enzyme to another nonreducing fructose with a free

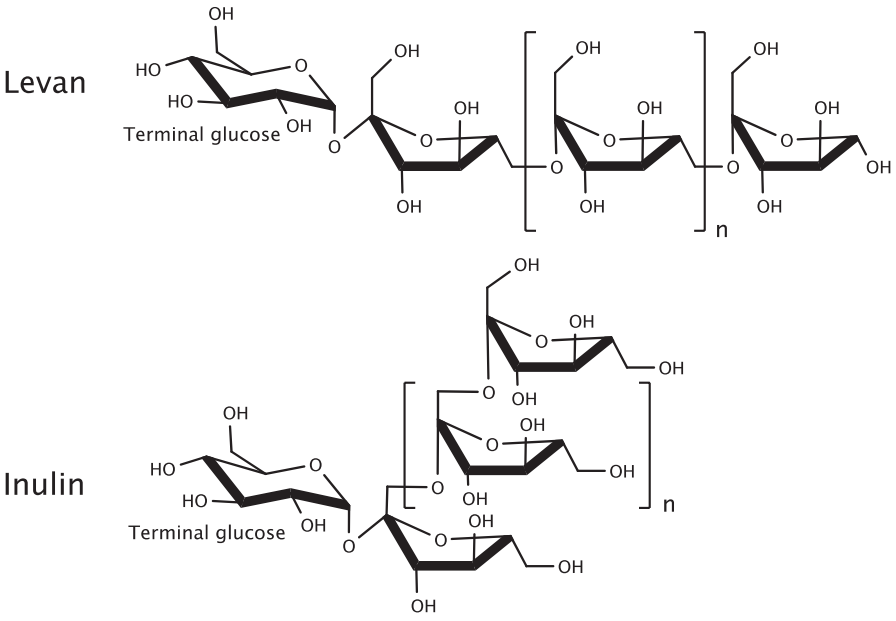

Fig. 4.4 Structure of levan and inulin synthesized by fructansucrase from sucrose. When sucrose is used as the acceptor in the initial priming reaction, synthesized fructans contain a nonreducing glucose unit at the end of the chain (terminal glucose)

primary alcohol at position C2, acting as an acceptor substrate, e.g., sucrose, raffinose, or a fructan molecule [87, 88]. This is also referred to as the priming reaction. In subsequent steps, the enzyme elongates the primer. A clear difference between fructansucrase and glucansucrase enzymes is the fact that glucansucrase enzymes cannot use sucrose as an acceptor but rather the cleaved glucose residue. The molecular masses of the fructans produced show a large variation, from 2×10^4 to 50×10^6 Da [28]. There are some reports that the molecular mass of the fructan produced is dependent on incubation conditions, the temperature, salinity, and sucrose concentration [89–91]. Sucrose analogues with a similar glycosidic linkage to sucrose have been used for the synthesis of new poly- and oligosaccharides by fructansucrase. For example, a wide range of fructansucrases recognize most of the sucrose analogues, such as those which were composed of galactose, mannose, xylose, fucose, and rhamnose in place of glucose, giving rise to novel poly- and oligosaccharides [92–97].

4.4 In Vitro-Synthesized α-Glucan as New Bio-Based Materials

The usage of naturally obtained mutan in the material application had not attracted any attention until now as the mutan has a branched structure; they lack a uniform composition, and purification costs are relatively high. The GtfJ enzyme, a kind of glucansucrase from *Streptococcus salivarius*, can effectively catalyze the one-pot water-based enzymatic polymerization of linear α(1 → 3)-glucan without branches. The synthesis process is environmentally friendly with a reaction in water medium, without organic solvent, and also convenient: only mixing sucrose solution with enzyme and storing at designated temperature. In addition, the molecular weight of the mutan can be adjusted by reaction conditions. Thus, in vitro-synthesized mutan can be considered as a potentially low-cost polymer for future prospect. A disadvantage of mutan, like all other polysaccharides, is the insolubility against most organic solvents and its thermally unprocessability. Up until now, many researchers have been attempting to improve this drawback by various techniques. One interesting method is the introduction of acyl groups to the hydroxyl groups of sugar units, or esterification, which raised the thermoplastic properties of cellulose known as so-called cellulose acetate and also improved the thermal properties and solubility of other polysaccharides in reported researches recently.

The series of mutan ester derivatives with Mw of about 200 kDa having different acid chain length were synthesized, and their thermal and mechanical properties of mutan ester films were investigated [58]. Hopefully, this material could be a substitution product to come on board replacing petroleum-derived plastics so as to avoid the uncertainty and sensitivity from oil's booms and busts, mitigate the environmental issues, and create the sustainability for future generations. Glass transition and melting behaviors of mutan ester series from C2 (acetate) to C8 (octanoate) are

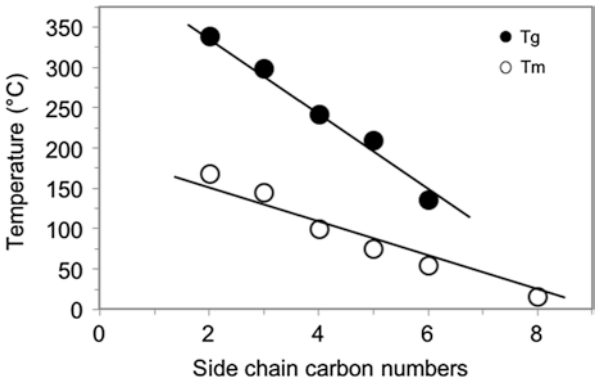

Fig. 4.5 Glass transition (T_g) and melting temperature (T_m) of mutan esters series from C2 (acetate) to C8 (octanoate). Redrawn on the basis of the reference [58]

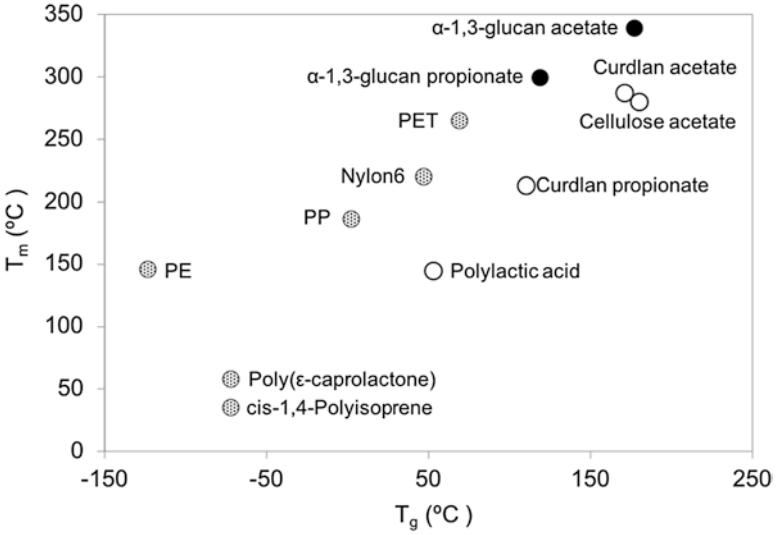

Fig. 4.6 Comparison of glass transition (T_g) and melting temperature (T_m) between those of mutan acetate and propionate, esters of other polysaccharides, and commercially available polymers. PE, PP, and PET are polyethylene, polypropylene, and polyethylene terephthalate, respectively. Redrawn on the basis of the reference [57]

shown in Fig. 4.5. The thermal and mechanical properties of mutan esters and its degree of crystallinity can be controlled by changing the length of its ester chain. Figure 4.6 shows the superior melting and glass transition temperature of mutan acetate and propionate over commercially available petroleum-based thermoplastics and currently interesting bio-based polymers; thus these materials are regarded as promising candidates for future thermoplastic application [57]. It was showed that unbranched mutan can be conveniently produced by green method using sucrose as a low-cost material. Furthermore, fully substituted mutan esters which

their thermal and mechanical properties can be adjusted are of interest for developing new thermoplastic materials.

4.5 Conclusions

Sucrase-type (non-Leloir-type) enzymes can catalyze to produce both polysaccharides and oligosaccharides using sucrose as a renewable cheap substrate via one-pot water-based reaction. The sucrase-type enzymes can be a very efficient tool to provide the synthesis of tailor-made glucan, fructan polysaccharides, and oligosaccharides for a wide range of applications. Several enzymatic processes with these enzymes already have been established for polysaccharide and oligosaccharide synthesis, some of which are applied industrially for a variety of applications. Further breakthroughs in this field are expected in the future, with enzyme engineering approaches increasingly allowing new construction of mutant enzymes and the discovery of new types of sucrase enzymes.

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