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Journal of Molecular Catalysis B: Enzymatic 10 (2000) 117–128

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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Glucansucrases: molecular engineering and oligosaccharide synthesis

Magali Remaud-Simeon, René-Marc Willemot, Patricia Sarçabal, Gabrielle Potocki de Montalk, Pierre Monsan *

Centre de Bioingénierie Gilbert Durand, UMR CNRS 5504, UR INRA 792, INSA, 135 Avenue de Rangueil, 310077 Toulouse Cedex 4, France

Received 20 July 1999; accepted 28 October 1999

Abstract

Due to their informative role in biological systems, the potential development of oligosaccharide utilization is very important. Today, their industrial application is increasing rapidly especially for their capability of specific stimulation of beneficial bacteria. Future development will require the access to specific oligosaccharides synthesized via processes compatible with technical and economical industrial constraints. In this context, glucansucrases are attractive tools allowing the production of different glucooligosaccharides (GOS) from simple substrates such as sucrose and maltose. These bacterial enzymes are responsible for the synthesis of glucan polymers. They can also synthesize oligomers when an acceptor molecule is introduced into the medium. A large variety of glucosidic bonds are formed corresponding to variable regiospecificities dependent on the enzyme origin. More than 30 glucansucrase-encoding genes have been cloned and sequenced. Many data were provided from studies on the structure/function relationship on these sucrose-converting glucosyltransferases (GTF). Sequence alignment analysis allowed identification of essential amino acids and clearly showed analogies with enzymes from the large α-amylase family. It is now possible to list some determinants possibly involved in the glucansucrase specificity, but many additional investigations and data, especially about the three-dimensional structure, will be necessary for the rational design of specific enzymatic tools for GOS synthesis.

Keywords: Dextransucrase; Glucosyltranferases; Amylosucrase; Oligosaccharides; Polysaccharides

1. Introduction

Oligosaccharides were traditionally used in food as a source of energy or as sweeteners. Nowadays, the knowledge concerning their informative biological function and their role in cell-surface interactions is increasing rapidly and greatly stimulates the field of glycotechnology. Besides their traditional use, oligosaccharides find more and more new applications in food, feed, pharmaceutical, or cosmetic industries as stabilizers, bulking agents, immunostimulating agents or prebiotic compounds able to stimulate

* Corresponding author. Tel.: +33-561-559-415; fax: +33-561-559-400.

E-mail address: monsan@insa-tlse.fr (P. Monsan).

1381-1177/00/$ - see front matter
PII: S1381-1177(00)00119-3

the growth of beneficial bacteria [1]. However, the development of this field is limited by the difficult access to a wide variety of original oligosaccharide or glycoconjugate structures. The multi-step chemical synthesis appears tedious compared to the enzymatic approach, which has been demonstrated to be much more selective. However, most of the biological reactions of glycoconjugate synthesis involve expensive nucleotide-sugar derivatives. To overcome this problem, glucansucrases really emerge as very attractive enzymes, which use sucrose as a simple and widely available substrate.

The primary physiological function of glucansucrase consists of synthesizing high molecular weight glucans [2]. However, the enzymatic activity can be redirected from glucan synthesis toward oligosaccharide synthesis when an efficient acceptor is added to the reaction mixture [3]. In that case, the enzyme catalyzes the transfer of the glucose from sucrose unto acceptor molecules. A large variety of glucansucrases, which synthesize different types of osidic bonds, is available [4]. As part of our research directed toward the enzymatic synthesis of oligosaccharides having dietary or prebiotic properties, we have studied the synthesis of oligosaccharides having α-1,2 osidic bonds catalyzed by the glucansucrase from *Leuconostoc mesenteroides* NRRL B-1299 and provided a useful method for producing these oligosaccharides on an industrial scale (40 t/year) [5].

To extend this concept to the synthesis of new and original glucoconjugates, it is necessary to have a better understanding of the mechanism of the acceptor reaction. Mechanisms for dextransucrase have been proposed by Robyt et al. [6] and Mooser [7]. There is a need to extend the study of these reactions to the molecular level in order to define the protein determinants involved in the selectivity towards a given acceptor molecular structure and in the regiospecificity of the enzyme. This could envisage the rational design of glucansucrases with both controlled selectivity and regiospecificity to be envisaged.

In this paper, we will focus on the variety existing among sucrose glucosyltransferases (GTF) (transglucosidases) useful for oligosaccharide synthesis and will examine the molecular information about glucansucrases that could be related to their specificity or selectivity.

2. Variety of glucansucrase products

Glucansucrases are sucrose converting GTF responsible for the synthesis of extracellular bacterial polysaccharides. Different kinds of glucans, with different types of linkages, different sizes and structures, depending on the glucansucrase-producing strain, are synthesized (Table 1).

2.1. Polysaccharides

Among these polysaccharides, dextran is the generic term given to a group of bacterial polysaccharides composed of chains of D-glucosyl units predominantly connected by α-1,6 linkages, with varying amounts and arrangements of α-1,2, α-1,3, α-1,4 linkages [4]. Dextrans are produced by various species of the genera *Leuconostoc*, *Lactobacillus* and *Streptococcus*. Dextransucrase from *L. mesenteroides* NRRL B-512F synthesizes a linear water-soluble dextran with 95% α-1,6 linkages in the main chains and 5% α-1,3 branch linkages. Some strains produce two different dextrans. *L. mesenteroides* NRRL B-1355 produces a typical linear dextran and another glucan named alternan composed of glucosyl residues linked by alternating α-1,6 and α-1,3 linkages. *L. mesenteroides* NRRL B-742 produces a dextran with main chains composed of α-1,6 linked glucosyl residues, highly branched with single glucosyl residues attached by α-1,3 linkage to every glucosyl unit in the main chain. *L. mesenteroides* NRRL B-1299 produces dextrans with α-1,2 linkages, this kind of osidic linkage being relatively rare in nature.

Table 1
Percentage of osidic linkages in the polysaccharide structure of the glucan produced by different bacterial strains

		Linkages				
		% α-1,6	% α-1,3	% α-1,2	% α-1,4	Reference
L. mesenteroides	NRRL B-512F	95	5			[8]
	NRRL B-742	87			13	[8]
		50	50			[8]
	NRRL B-1299	66	1	27		[8]
		65		35		[8]
	NRRL B-1355	95	5			[8]
		54	46			[8]
S. mutans GS5	GTF-I	13	87			[9]
	GTF-IS	15	85			[10]
	GTF-S	70	30			[11]
S. downei	GTF-I	12	88			[12]
	GTF-S	90	10			[13]
Amylosucrase		6			94	[14]

Streptococcal strains can produce glucans composed of less than 50% of α-1,6 linkages and more than 50% of α-1,3 linkages. These polysaccharides are named mutans. They can be differentiated from dextrans by their structure and physical properties but the enzymes responsible for the synthesis of both dextrans and mutans are very similar. Streptococcal dextransucrases and mutansucrases are generally known by the generic term of GTFs. Mutan is a very water-insoluble glucan playing an essential role in cariogenesis by enhancing the attachment and colonization of teeth surface by streptococcal cariogenic bacteria [15].

Amylosucrase is a GTF that uses sucrose to produce a linear polymer composed of α-1,4 glucopyranosyl residues having similarities with amylose [14,16]. This enzyme was shown to be excreted by *Neisseria perflava* and *Neisseria polysaccharea*. Amylosucrase can produce an amylose-like polymer without primer, but sucrose consumption rate is increased in the presence of glycogen, some polymer branchings being linearly elongated with α-1,4 linked glucopyranosyl residues [17].

Isolation of genes coding for glucansucrases allowed individual characterizations of the enzyme activities. Most of the strains contain several glucansucrase-encoding genes. Proteins expressed from these genes catalyse the synthesis of glucans with different structures confirming the observations made on the wild strains. Moreover, the production of several different glucans by some bacteria can generally be correlated to the expression of different genes [18].

2.2. Oligosaccharides and products of acceptor reactions

Although glucansucrases accept a limited number of substrate donors other than sucrose, numerous sugars can act as acceptors [3,19]. Some acceptors, such as disaccharides like maltose or oligosaccharides presenting an isomaltosyl residue at their non-reducing end, allow a series of oligosaccharides to be produced [20]. Synthesis progresses by successive transfers of glucosyl units to oligosaccharides, which can be alternatively product and substrate. Other acceptors, like fructose [21], allow only the production of a disaccharide (leucrose), which is not an acceptor. The leucrose synthesis reaction increases at the end of the glucan synthesis reaction when fructose has accumulated in the medium. A process of leucrose production by action of dextransucrase in the presence of sucrose and fructose has also been developed on a pilot-scale [22].

A variety of enzyme specificities, close to that observed in high molecular weight glucan synthesis, is also observed during the oligosaccharide synthesis by acceptor reaction. The glucooligosaccharides (GOS) produced by the action of dextransucrase from *L. mesenteroides* NRRL B-512F on maltose and sucrose, are composed of maltose residue located at the reducing end and additional glucosyl residues all α-1,6 linked [20,23].

Using dextransucrase preparation produced by *L. mesenteroides* NRRL B-742, Remaud et al. obtained a mixture of linear GOS identical to oligosaccharides produced by the B-512F dextransucrase and α-1,3 branched oligosaccharides [24].

Oligosaccharide production using alternansucrase from *L. mesenteroides* NRRL 1355 was also investigated. In the presence of maltose and sucrose, oligoalternan is synthesized [25,26]. In fact, as shown by action on isomaltose, the transfer onto a glucosyl residue already α-1,6 linked to another glucosyl residue preferentially results in the formation of an α-1,3 bond, α-1,6 linkages being formed later in the reaction [25].

Synthesis of GOS using dextransucrase from *L. mesenteroides* NRRL B-1299 was studied in detail by Dols et al. [27]. Three families of homologous molecules were observed (Fig. 1). The first family consisted of oligosaccharides identical to GOS produced by the B-512F dextransucrase. The second family was composed of linear oligosaccharides containing α-1,6 linked glucopyranosyl residues and an α-1,2 linked terminal residue located at the non-reducing end. The third family was formed of oligosaccharides containing a linear chain composed of α-1,6 linked residues and α-1,2 branched residues. Oligosaccharides containing α-1,2 linked residues are highly resistant to the attack of digestive enzymes. These industrial products are used as prebiotics in cosmetic and human nutritional applications, as they are specifically metabolized by beneficial saprophyte flora [28].

Dols has optimized the production of GOS containing α-1,2 linked residues [29]. For GOS production, the two essential parameters were the sucrose concentration and the sucrose/maltose ratio (S/M). The optimal values were a sucrose concentration equal to 45% (w/w) and

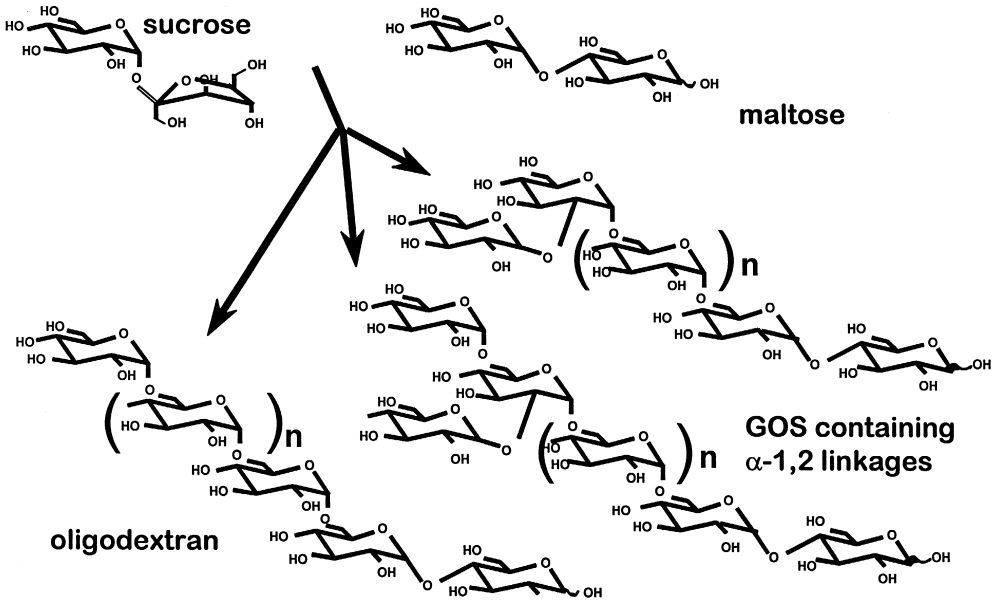

Fig. 1. Variety of oligosaccharides produced by *L. mesenteroides* NRRL B-1299.

a sucrose/maltose ratio of 4. At low S/M values, the oligosaccharide production is favored. Low levels of polymer of dextran and leucrose (undesirable by-products) are obtained but maltose generally is not totally consumed. In contrast, high S/M ratios resulted in the production of oligosaccharides that are larger in size and in an important synthesis of high molecular weight polymer and leucrose. Fig. 2 shows the composition of the GOS mixture obtained under optimized conditions: 55% of the oligosaccharides contained α-1,2 linked residues and a major part of the GOS had a degree of polymerization of 5 and 6.

In this context, to enlarge the applications of glucansucrases for practical purposes in particular for high production yield of glucoconjugates of controlled structure, it is necessary to have access to enzymes with controlled selectivity and regiospecificity toward acceptors.

In the presence of good acceptors (such as maltose), synthesis of what must be considered as by-products, such as high molecular weight glucan or leucrose, is very limited but with acceptors of poor efficiency, by-product synthesis is a real problem and limits the production yield of oligosaccharides. There is thus a need to engineer glucansucrases in order to facilitate the recognition of poor acceptors and limit the by-product formation. Equally, the spectrum of acceptors likely to be glucosylated by glucansucrase, which is already broad, could probably be enlarged. The other target is to identify the glucansucrase determinants that define the specificity of each enzyme. One very important point is the fact that glucansucrases keep their specificity when they catalyze oligosaccharide synthesis in the presence of an acceptor. It has been demonstrated that the enzyme presents at least two different sites: a sucrose binding site and an acceptor-binding site [30–32]. Competitive inhibitors bind to the sucrose-binding site and non-competitive inhibitors bind to the acceptor-binding site. Detailed structural information of glucansucrase–acceptor interaction is necessary to localize the acceptor binding and to determine the variations in glucansucrases that are responsible of their specificity.

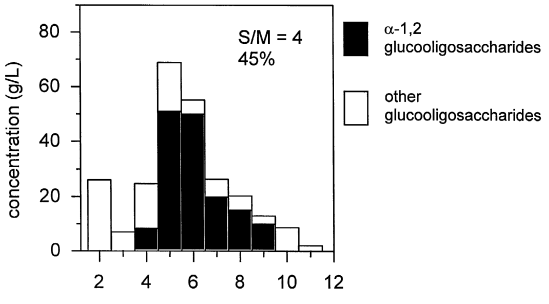

Fig. 2. Composition of GOS mixture produced by dextransucrase from *L. mesenteroides* NRRL B-1299. Initial sucrose content: 45% (w/w). Initial sucrose/maltose ratio: 4.

3. Key molecular determinants of glucansucrases

More than 30 genes encoding sucrose GTF (also named glucansucrase) are now available [9–13,17,33–42]. Recombinant amylosucrase, the glucansucrase from *N. polysaccharea*, purified by affinity chromatography [17], has been crystallized [43]. Crystals suitable for X-ray experiments have recently been reported and the elucidation of the three-dimensional structure of the enzyme is expected soon [43]. In spite of the importance of this knowledge, the crystallization of the enzymes from *Leuconostoc* and *Streptococcus* species still defies crystallographers probably because of the large size (between 155 to 200 kDa) and the flexibility of these proteins. In spite of the lack of structural data, investigations over the past decade combining biochemical studies, sequence alignment analysis, hydrophobic cluster analysis and structure predictions have revealed that sucrose GTF share many mechanistic and structural features with glycoside hydrolases and particularly with glucoside hydrolases like α-amylase, cyclodextrin glucano transferase or pullulanase which, on the basis of sequence alignment, have been

shown to belong to the same family 13 [44]. These advances are of importance because they open new routes for protein engineering attempts and for producing enzyme specific to one type of osidic bond and also highly specific to oligosaccharide synthesis without by-product synthesis.

3.1. Relatedness with enzymes from the α-amylase family (family 13)

3.1.1. The glucosyl-enzyme intermediate: a common initial step to sucrose GTF and glucoside hydrolases

Using sucrose osidic bond as a source of free energy in glucosyl transfer to an acceptor, glucansucrases catalyse the synthesis of α-1,2, α-1,3, α-1,4 or α-1,6 glucosidic bonds (depending on their specificity) with retention of configuration of the anomeric carbon. The reaction begins with a nucleophilic substitution at the anomeric-saturated carbon [45–48]. From a mechanistic point of view, sucrose GTF appeared to be very similar to the glycoside hydrolases and they all possess a sometimes weak, but detectable, hydrolytic activity [49]. In agreement with this suggested mechanism, a D-glucosyl-enzyme intermediate has been isolated for several GTF from *Leuconostoc* or *Streptococcus* species using two different methods: the stabilization of a native glucosyl-enzyme immobilized on a chromatographic resin [46,50,51] and the denaturation of a steady-state complex of radiolabelled substrate and enzyme [52]. As expected, the chemical method allowed a β-glucosyl anomer ester of a carboxylic acid to be trapped. This carboxylic acid was further identified as an aspartic acid [53]. By means of site-directed mutagenesis, replacement of the corresponding aspartic acid by asparagine further confirmed that this amino acid is essential for catalytic activity of sucrose GTF from *Streptococcus mutans* [54], *Streptococcus downei* [55], *L. mesenteroides* NRRL B-512F [56]. So, considering only the initial step of the reaction, glucansucrases are expected to be very close to glucoside hydrolases.

3.1.2. Predicted structure of the glucansucrases

Considering the group of glucansucrases from *L. mesenteroides* and *Streptococcus* sp., a high overall degree of identity is encountered within the sequences of this glucansucrase group which, in other respects, present distinct characteristics such as the need for a glucan primer, the variable activation by exogenous dextran, the structure and size of the glucan produced, the affinity towards various types of acceptor. On the basis of genetic, biochemical and structure and functional studies, four regions have been identified in these proteins: a signal peptide, a variable region and an N-terminal catalytic domain followed by a C-terminal glucan binding domain (GBD) (Fig. 3) [36,38,57–59]. As shown in Fig. 3, all the GTFs possess at the N-terminal extremity a signal peptide region of 35 to 38 amino acids with more than 50% identical or functionally equivalent residues except DSR-A from *L. mesenteroides* NRRL B-1299 [34]. The signal peptide region is followed by a highly variable region of 140 to 261 amino acids in which only a few amino acids are conserved. The longest variable region is encountered in DSR-S from *L. mesenteroides* NRRL B-512 F and ends at the consensus sequence ^{268}GxxYYxDxxG^{277} common to all the glucansucrases (Fig 3). The amino acid sequence role of this region has not been extensively investigated yet. It has been suggested that this region could be a tracer specific for each GTF because of its high variability [60]. On the other hand, the high degree of variability implies that it may not have an important function in the protein. In fact, *DSR-A* gene from *L. mesenteroides* NRRL B-1299 does not possess this variable part and is still active. Moreover, an active catalytic core, not including this part of the N-terminal sequence, has been isolated from the GTF-I of *S. downei* [61]. Immediately following the variable region, these proteins possess a highly

conserved region of about 900 amino acids (shown on Fig. 3), named the N-catalytic region because it contains the essential aspartic acid involved in the glucosyl-enzyme intermediate [53,54]. The structural relatedness of this region with the enzyme from the α-amylase super family was first suggested by Ferreti et al. [36]. Although it was impossible to align the overall sequence of these glucansucrases with the enzymes from family 13, short stretches of amino acids identical to very conserved segments in enzymes from family 13 were found along the N-terminal catalytic domain of glucansucrases [55,62]. Following this, two structure predictions have been proposed for the glucansucrases from *L. mesenteroides* and *Streptococcus* sp. that confirm the relatedness between this glucansucrase group and the enzymes from family 13. Indeed, both predictions concluded that these enzymes possess a catalytic $(\beta/\alpha)_8$ barrel domain. But, whereas Devulapalle et al. concluded that glucansucrases from *Streptococcus* and *Leuconostoc* sp. are members of the family 13 and possess a classical catalytic $(\beta/\alpha)_8$ barrel domain [55], Mac Gregor et al. concluded that the (β/α) domain is circularly permuted [62] (Fig. 3). Besides, the two models come to an agreement for designation of helix and β-strand 3 to 8, but the model proposed by Devulapalle would not fit for DSR-A from *L. mesenteroides* NRRL B-1299, the sequence proposed for β-strand 1 to helix 2 being absent in this protein. Consequently, in the new classification based on amino acid sequence similarities available on WWW server, the glucansucrases from *Leuconostoc* species and from *Streptococcus* species have all been gathered into family 70 of the glucoside hydrolases [44,63].

Among the sucrose GTF, amylosucrase emerges as a unique enzyme. It is much smaller

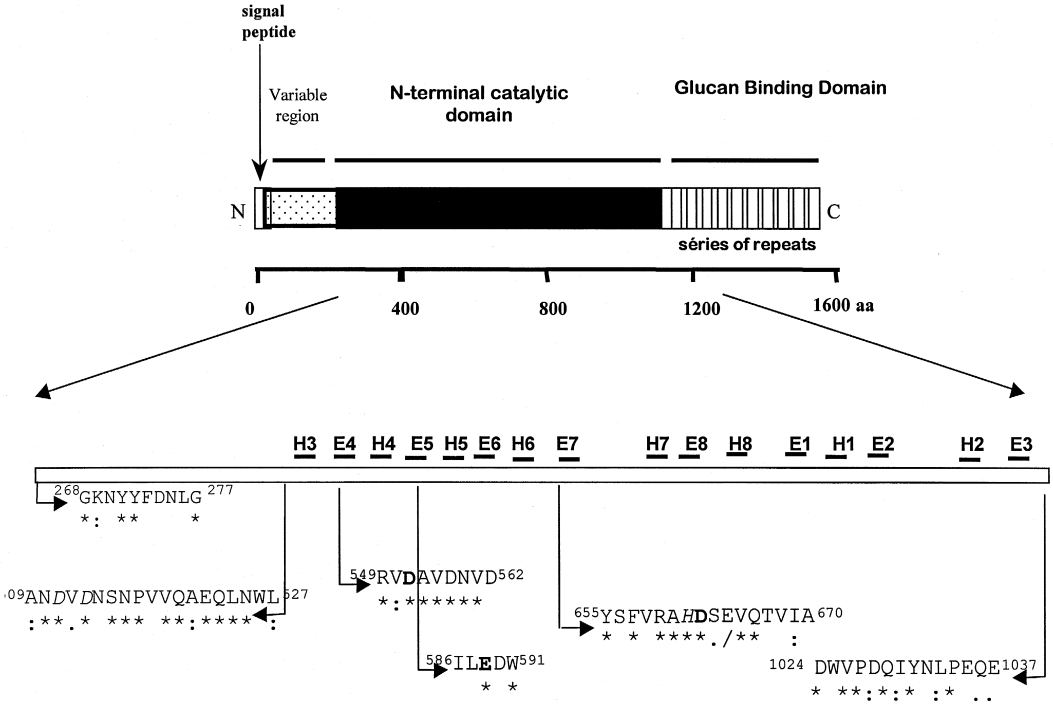

Fig. 3. General diagram of glucansucrase from *L. mesenteroides* NRRRL B-512F (DSR-S). The invariant amino acids (*) in the consensus sequence with the other glucansucrases from *S. mutans* and *L. mesenteroides* are indicated. Catalytic triad residues are given in bold characters. Other residues suspected of being involved in catalysis are indicated in italic.

in size (636 amino acids) than the sucrose GTF from *Leuconostoc* and *Streptococcus* sp. (1250 to 1600 amino acids). In addition, structure prediction clearly showed that this enzyme belongs to the large family 13 of the glucoside hydrolases [17] with a catalytic $(\beta/\alpha)_8$ barrel domain like α-amylase, pullulanase or cyclodextrin glucanotransferases.

These predictions have allowed the localization, in the sequence of glucansucrases, seven of the eight conserved regions that serve as fingerprints in α-amylase family [17]. The three carboxylic acids: D551, E588,and D662 of DSR-S (equivalent to D295, E 336 and D401 of amylosucrase) known to be involved in the glucosyl bond cleavage for enzymes from family 13 are conserved in glucansucrases. Their individual replacement by their amide derivatives in GTF I from *S. downei* and in amylosucrase produces mutants with almost no activity [55,64]. H195 and H400 of amylosucrase, which are suspected of stabilizing the glucosyl-enzyme intermediate in α-amylase [65,66], have been changed to a glutamine and an asparagine, respectively, and H661 of DSR-S [56] and H561 of GTF-B (equivalent to H400 of amylosucrase) [67] have been replaced by an arginine. The resulting mutant proteins display a considerably reduced activity. These effects are in agreement with the proposed structure predictions and further support the similarity between the α-amylase and glucansucrase catalytic mechanism. By analogy with α-amylase and CGTase, E336 of amylosucrase (and equivalent residues in other glucansucrases) can be proposed as the general catalyst. D294 (amylosucrase numbering) would participate to the formation of the glucosyl-enzyme intermediate and D401 would be involved in the distortion of the sucrose glucosyl ring [66,68].

The relatedness between glucansucrases and glucoside hydrolase of family 13 already give new insights into envisaging with more accuracy enzyme modeling and identifying sequence motifs, which may be associated with specificity. Although precise details of the structural determinants, which define the specificity, are still limited for the enzyme from family 13, the body of knowledge is increasing rapidly. Several amino acids in the areas close to the substrate binding-site have been found to modulate the substrate or product specificity [69–71].

3.2. Determinants possibly involved in glucansucrase specificity

3.2.1. Area close to the second aspartic acid of the catalytic triad

To address the problem of the relations between glucansucrase specificity and glucansucrase structure, Shimamura et al. identified amino acid variation between the glucansucrases that synthesize an α-1,3 linked glucan (GTF-I) and those which are more specific of α-1,6 glucosidic bond synthesis (GTF-S) [72]. Six conserved positions were selected. The amino acids conserved in GTF-I were replaced individually or in combination by the equivalent amino acid found in GTF-S of *S. mutans* GS-5. From this study, it appears that the presence of a carboxylic acid instead of a neutral one (threonine) at the position 589 of GTF-S (corresponding to T667 of DSR-S) increased by 30% the synthesis of α-1,3 glucosidic bond. Consistent with these results, DSR-S T667R mutant was found to synthesize a glucan with 13% α-1,3 linkages compared to less than 5% for DSR-S. In addition, oligosaccharide yield in the presence of maltose or fructose acceptor was significantly increased [73]. T667 of DSR-S (equivalent to T589 of GTF-S) is close to the second aspartic acid involved in the catalytic triad of enzymes from α-amylase family where it would exert distortion on the glucosyl ring (Fig. 3). Although it is not possible, without structural data, to clearly determine the role of the amino acids in the vicinity of the aspartic acid, it is likely that these residues also exert an effect on glucosyl residue or on acceptor placement in glucansucrases.

3.2.2. GTf-P1 region

Another important functional region of 19 amino acids corresponding to the following sequence ^{509}ANDVDNSNPVVQAEQLNHL^{527} in DSR-S and ^{262}QWDLNYSNPWVFRAMAGEM^{280} in amylosucrase also plays an important role in glucansucrases, although it seems difficult to clearly establish its function. In fact, insoluble glucan synthesis is inhibited by antibodies directed against this sequence (named Gtf-P1), while the soluble one is not affected [74] and this peptide was recently demonstrated to be one of the major B-cell epitopes in the human humoral immune response [75]. Funane et al. [76] concluded from an enzyme deactivation by a chemical agent active on carboxylic groups, which is delayed in the presence of sucrose monocaprate that it could be another sucrose binding site. In addition, this region resembles a calcium binding region of some enzymes from family 13 and homology of sequence between amylosucrase and DSR-S is quite evident. Furthermore, site-directed mutations of the two aspartic acids present in GTF-P1 have shown that both of them are important for catalysis but not to the same extent. Moreover, there could be a relation between the structure of the glucan produced and the role of these aspartic acids. In GTF-B (which synthesizes an α-1,3 linked glucan), Asp 411 and 413 (equivalent to D511 and D513 of DSR-S) have been individually replaced by either a threonine [67] or an asparagine [77]. GTF-B D413N and D413T retained 20% and 12% of activity, respectively. D411N still retained 20% of activity, whereas D411T mutant was not affected by the mutation. Equivalent mutations in GTF-C from *S. mutans* (which synthesizes a polymer with a majority of α-1,6 linkages) at positions 437 and 439 resulted in a complete loss of activity. Consistent with this finding, DSR-S D513N mutant retained only 4% of activity compared to the wild enzyme and no activity was detectable for DSR-S D511N mutant. Consequently, these aspartic acids seems to be more important for the enzymes that synthesize a polymer with high degrees of α-1,6 linkages than for those which synthesize a polymer with predominantly α-1,3 linkages. However, it must be emphasised that the enzymes were not purified to the same extent and that the sensitivity of the assay was not always comparable. Further investigations are needed to confirm the functional or structural role of these residues and to demonstrate that they may function differently depending on the specificity of the glucansucrase.

3.2.3. GBD

Finally, at the carboxy terminal, glucansucrases from *Streptococcus* species or *L. mesenteroides* strains possess a long GBD [36,39,57,78] that contains different homologous direct repeats named A, B, C D and N repeat on the basis of their sequence similarity [36,57,79,80]. The organization and number of the repeats vary in the various glucansucrases. Although GBD is not directly involved in either sucrose cleavage, glucan synthesis or oligosaccharide synthesis, its presence modulates the initial rate of the reaction [36,39,54,81]. But again, differences are observed among glucansucrases, the enzyme synthesizing predominantly α-1,3 linkages being less affected by deletions at the carboxy terminus than the enzyme synthesizing α-1,6 linkages. Recently, a core region has been engineered from GTF-I of *S. downei* with 546 amino acids at the C-terminal end (including all the repeats) deleted [61]. The core retained more than 70% of activity, whereas a deletion of only one A repeat in DSR-S led to 94% decrease in initial sucrose consumption rate [61,80]. Moreover, the structure of the glucan and the oligosaccharides produced by deleted enzymes was comparable to the structure of the glucan and the oligosaccharides synthesized by native enzymes [80]. These findings are consistent with a study carried out with hybrid GTF-B and GTF-C *S. mutans* enzymes, which indicates that the repeat region is not directly involved in the solubility of the glucan produced [82]. In contrast, a spontaneous mutant deleted of some repeats at the C-terminus

end of GTF-G was found to produce a higher percentage of α-1,6 linkages in the glucan polymer than the wild-type enzyme [83].

Although it seems that the structural features that determine the specificity of the glucansucrases are likely to be located in the N-catalytic domain of glucansucrase, it seems to be too early to conclude that GBD has no role at all in enzyme specificity. In fact, the oligosaccharide size distribution is modified when the C-terminal domain is partially deleted [80].

4. Conclusion

Although numerous genes encoding glucansucrases are available, information on the determinants that are involved in their selectivity and regiospecificity remains limited. It is likely that a complex molecular machinery is at the origin of the specificity. To gain further insight at the molecular level, it is of importance to possess the sequence of very uncommon enzymes among glucansucrases such as dextransucrase from *L. mesenteroides* NRRL B-1299 that synthesizes α-1,6 and α-1,2 glucosidic bonds, alternansucrase from *L. mesenteroides* NRRL B-1355 that synthesizes a polymer with alternating α-1,3 and α-1,6 linkages or dextransucrase from *L. mesenteroides* NRRL B-742, which catalyzes a comb-like dextran with alternating α-1,3 and α-1,6 linkages. Sequence analysis will enable distinct amino acids or motifs putatively associated with specificity to be identified and their role by means of site-directed mutations to be investigated further. Of course, the rational deign of glucansucrases specific for oligosaccharide production will undoubtedly be accelerated when three-dimensional structures are available and when soaking experiments in the presence of acceptor or non-competitive inhibitor enable the acceptor binding site to be localized precisely. Finally, the number of genes already available coding for enzymes with distinct specificities makes glucansucrase genes very good candidates for shuffling experiments. This approach has also to be considered to gain further insight into the enzyme determinants involved in the specificity. This will enable oligosucrases of controlled specificity with efficient catalytic properties to be produced.

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