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REVIEW

Oligosaccharide Synthesis by Enzymatic Transglycosylation

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Introduction

Saccharides encompass a wide variety of carbohydrate-containing compounds. These include polysaccharides, oligosaccharides, glycoproteins and glycosides with non-carbohydrate aglycones. Biological macromolecules composed of protein, lipids or nucleic acids containing oligosaccharides are collectively known as glycoconjugates. The carbohydrate moiety can provide many biological functions, such as stability [1], alteration of macroscopic physical properties [2] and immunogenic response [3]. An interesting natural function of oligosaccharides is seen in the Galβ1-3GalNAc glycoprotein, which imparts substantial anti-freeze properties on antarctic fish serum [4].

While the biosynthesis of glycosides with non-carbohydrate aglycones is a widespread phenomenon in plants, animals and microbes, this topic is beyond the scope of this review. The reader is referred to recent reviews of the biochemical function of glycoconjugates [5] and the biosynthesis of *N*-linked and *O*-linked oligosaccharides in glycoproteins [6-8].

Polysaccharides and their biosynthesis have also been well-studied and are the subject of many books and reviews [9-15]. These enzymatic processes involving polysaccharide synthesis have been well-documented and will not be reiterated here.

Oligosaccharides differ by monosaccharide structure, composition, sequence and linkage. Many types of oligosaccharides formed by enzymatic and chemical means have been catalogued [16, 17]. Some enzymatically produced oligosaccharides have been produced

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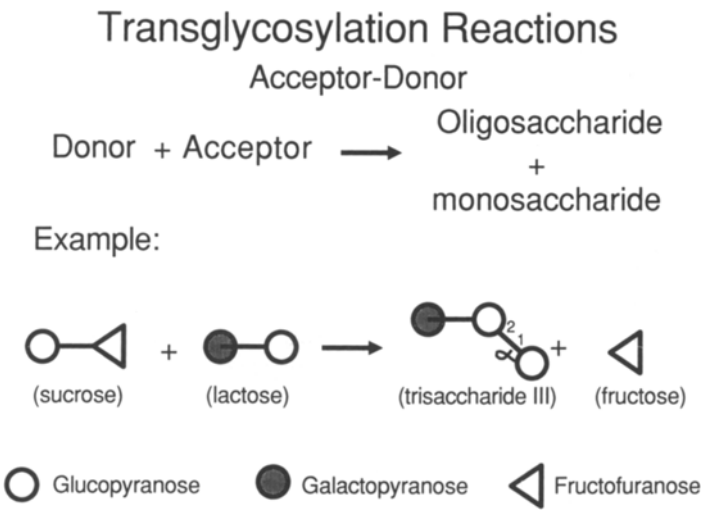

Figure 1. Transglycosylation reactions utilizing sucrose as the glycosyl donor. Structures are represented according to the system proposed by Robyt [121].

commercially. A well known example is the cyclodextrin group of saccharides and their derivatives [18]. These cyclic maltodextrins are known for their ability to complex numerous hydrophobic substances, thereby rendering them water-soluble. Another example is the group of D-fructofuranosyl sucroses known under the trade name of Neosugar. These have great potential as low-calorie, non-cariogenic sweeteners [19, 20]. Our intention in this review is to describe the direct formation of oligosaccharides by enzymic transglycosylation.

Glycosyl Transfer Reactions

There are several different types of transglycosylation reactions, differing mainly in the type of glycosyl donor used. The most common type is the transfer of a glycosyl unit from a sugar nucleotide to an acceptor, such as a growing oligosaccharide chain. This is the mechanism of formation of the oligosaccharide portions of glycoproteins, for example.

Nucleotide sugars also act as the initial donors in the biosynthesis of most polysaccharides, which proceeds through lipid-linked oligosaccharide intermediates. This type of transglycosylation has been reviewed recently [13, 21] and is discussed in many textbooks. Many types of protein-oligosaccharide transferases mediate these reactions, depending on the various amino-acid and oligosaccharide structures. A discussion of these oligosaccharide transferases has previously been presented by Sadler [7] and Snider [8]. We will instead discuss more unusual types of transglycosylation reactions.

Oligosaccharide formation by enzymatic transglycosylation was the subject of a review over thirty years ago [22]. Since that time, there has been a dramatic increase in both the number and types of known reactions of this sort. A general review of the various mechanisms of transglycosylation reactions has been presented [23]. One type of transglycosylation involves the transfer of glycosyl units from a disaccharide to another carbohydrate acceptor. The most common disaccharide donor in these types of reactions is sucrose (see Fig. 1).

The glucopyranosyl-fructofuranosyl linkage is a relatively high-energy bond and so it is not surprising that sucrose is often used as a donor in oligosaccharide and polysaccharide biosynthesis. These enzymes include the glycansucrases, such as dextransucrase, levansucrase and amylosucrase. A review of some of the early work done with these enzymes appeared in 1951 [24]. Interestingly, despite the difference in product structure, there does appear to be some similarity in the substrate binding behavior of these enzymes [25], indicating a possible similarity in mechanism. Besides catalyzing the formation of polysaccharides, these enzymes also carry out what are referred to in the literature as acceptor reactions, whereby glycosyl units are transferred to an acceptor molecule bearing a hydroxyl group, most often another sugar.

Although other types of disaccharides besides sucrose and its homologues can act as glycosyl donors, the biosynthesis of polysaccharides from them is a thermodynamically unfavorable process. One exception worthy of mention is an enzyme from *Escherichia coli*, amylomaltase, which is capable of forming amylodextrins from maltose under certain circumstances [24].

Another well recognized phenomenon is the formation of oligosaccharides from other oligosaccharides by what are normally considered hydrolytic enzymes. Many enzymes are known primarily to catalyze the hydrolysis of oligosaccharides, but were also found to catalyze transglycosylation reactions. An example of such an enzyme is *Bacillus macerans* amylase, also known as cyclodextrin glucanotransferase (see Fig. 2). In addition to hydrolyzing starch to form linear and cyclic maltooligosaccharides, this enzyme also catalyzes a number of other reactions, such as disproportionation (the transfer of glycosyl groups from a donor to an identical acceptor, to give two new saccharides) and coupling [26] (see Fig. 3). In fact, it has been recognized for quite some time that the hydrolysis of the glycosidic bond is simply a special case of transglycosylation, in which the acceptor is water [22]. The relationship between enzyme-catalyzed glycoside hydrolysis and glycosyl transfer is discussed in some detail by Nisizawa and Hashimoto [27]. Theories of molecular mechanism of glycosyl group transfer have been detailed by Sinnott [28] and by Dedonder [23].

Another, related phenomenon is the enzymatic reversion reaction, or condensation, whereby free monosaccharides are linked together with the concomitant release of a water molecule (see Fig. 2). It almost goes without saying that any chemical reaction can proceed in either direction, depending on the thermodynamic equilibrium, and this applies equally to enzyme-catalyzed reactions. Thus, glycosidases are theoretically capable of forming as well as breaking glycosidic bonds. Needless to say, the thermodynamics of such a reaction are not very favorable, but under the proper conditions, hydrolytic enzymes can be induced to form glycosides, including disaccharides and sometimes higher oligosaccharides [29].

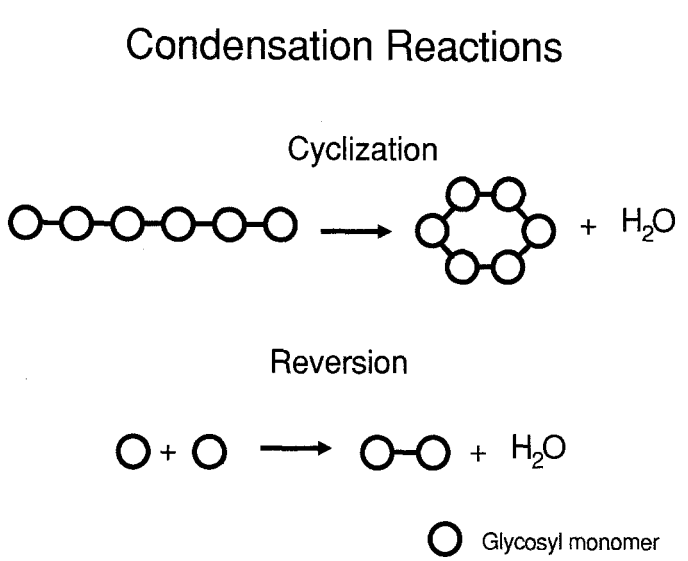

Figure 2. Condensation-type reactions, whereby glycosidic bonds are synthesized *de novo*. Structures represented as in Fig. 1.

Glycosides from Sucrose

D-Glucosyltransferases

Buchholz [30] has recently reviewed some enzymatic syntheses of oligosaccharides and polysaccharides from sucrose, and has discussed the general aspects of sucrose utilization by a variety of enzyme systems. Chemical sucrose analogues, such as α-D-glucopyranosyl fluoride, have also been shown to participate in glycosyltransferase reactions in a manner similar to sucrose [31]. We limit our discussion here to the so-called acceptor reactions of glycansucrases and related enzymes. These enzymes are responsible for the polymerization of one of the two monosaccharide components of sucrose and some of its analogues to give rise to such polysaccharides as dextran and levan. They also catalyze, in the presence of certain sugars or other suitable acceptors, the glycosylation of the acceptor to make a new di- or oligosaccharide.

It was noted early on that dextransucrase preparations from *Leuconostoc mesenteroides* could form oligosaccharides by glucosylation of acceptor sugars. This was first observed for the formation of the disaccharide leucrose by the glucosylation of D-fructose, which had been liberated from the donor, sucrose [32]. While sucrose is β-D-fructofuranosyl α-D-glucopyranoside, a non-reducing sugar; leucrose is the reducing sugar, α-D-glucopyrano-

syl-(1-5)-D-fructose. Later, a number of other sugars were also shown to act as acceptors for dextransucrase [33], including isomaltose, maltose, α-methyl D-glucopyranoside, D-glucose, D-fructose, melibiose and D-galactose. In the ensuing years, U.S.D.A. chemists determined the structures of some of the acceptor reaction products, which included isomaltodextrins from D-glucose and isomaltose [33], the corresponding α-methyl isomaltodextrins from α-methyl D-glucopyranoside [34] and panose and its higher homologues from maltose [35]. In addition to leucrose [36], another disaccharide, isomaltulose, also known as palatinose, was formed in lesser quantities from D-fructose [37]. Isomaltulose has the structure α-D-glucopyranosyl-(1-6)-D-fructose. The investigators also found that the yields of specific acceptor products could be increased by choosing the proper concentrations of donor and acceptor.

Others soon became interested in the acceptor reactions of dextransucrase and before long the enzymic syntheses of many interesting oligosaccharides were reported. Raffinose was found to act as an acceptor [38], giving rise to the branched tetrasaccharide I (see Fig. 4 for structures). The acceptor reaction with cellobiose was found to produce the trisaccharide II. Likewise, when lactose was the acceptor, the trisaccharide III was formed [39]. It was also discovered that when free D-galactose was added as an acceptor, a non-reducing disaccharide, β-D-galactofuranosyl α-D-glucopyranoside, was formed [40]. Hehre also found that *L. mesenteroides* dextransucrase was capable of synthesizing non-reducing sugars in the presence of certain acceptors. The first of these to be reported [41] was lactulosucrose (IV), formed from lactulose [β-D-galactopyranosyl-(1-4)-D-fructose] in the presence of sucrose. Interestingly, lactulosucrose can also act as a D-glucosyl donor for dextransucrase, one of the few saccharides capable of doing so [42]. Later, two more non-reducing disaccharides were found to be produced by an *L. mesenteroides* dextransucrase [43]. The acceptors were D-galactose and D-mannose, which gave rise to β-D-galactofuranosyl α-D-glucopyranoside (which Bourne *et al.* [40] had previously reported) and α-D-glucopyranosyl β-D-mannopyranoside, respectively. Yamauchi and Ohwada [44] tested all eleven of the possible glucose disaccharides and found that dextransucrase transferred D-glucopyranosyl units from sucrose to the 6-hydroxyl position of the non-reducing D-glucopyranosyl moiety of the disaccharide in all but two cases. α,α-D-Trehalose was unreactive, while cellobiose gave rise to the trisaccharide II, confirming the results of Bailey *et al.* [39]. Robyt and Walseth [45] have explained the mechanism of dextransucrase acceptor reactions within the framework of their reducing-end insertion mechanism for dextran biosynthesis. This model for dextransucrase acceptor reactions was further elaborated upon by Robyt and Eklund [46, 47].

While most of the work on dextransucrase acceptor reactions was done with enzymes that typically produce a D-glucan with α(1-6)-linkages and various branching points, there also exists a class of D-glucansucrases which synthesize D-glucans with alternating α(1-3)- and α(1-6)-linkages. One of these unusual D-glucansucrases, from *L. mesenteroides* NRRL B-1355, was isolated and its acceptor reaction products were studied [48]. It was found to produce, in addition to the expected α(1-6)-linked products, some unusual oligosaccharides containing both α(1-3) and α(1-6)-linkages.

Other organisms besides *L. mesenteroides*, including Streptococcus spp, also make D-glucansucrases. While their acceptor reactions have not been studied in as much detail as those from Leuconostoc spp., they are known to produce some of the same products [49].

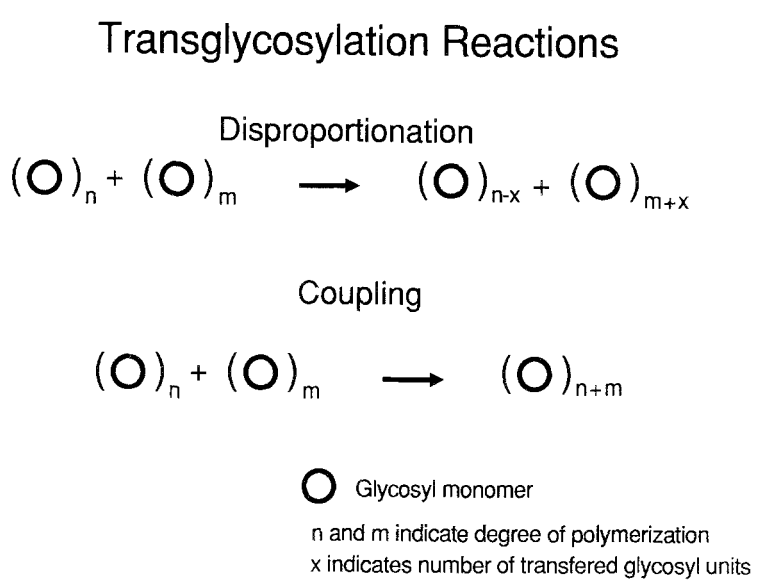

Figure 3. The types of transglycosylation reactions commonly referred to as disproportionation and coupling. Structures represented as in Fig. 1.

Mayer *et al.* [50] have reported on the acceptor specificity of *Streptococcus sanguis*, although the structures of the products were not determined. Other studies done on the acceptor reactions of streptococcal D-glucansucrases include that of Ono *et al.* [51], in which they found leucrose and other, unidentified saccharides to be synthesized. Binder and Robyt [52] studied the reactions of sucrose analogues with enzyme preparations from *Streptococcus sobrinus* 6715. They found that 3-deoxysucrose and 3-deoxy-3-fluorosucrose could act as glycosyl donors in acceptor reactions, to give the corresponding deoxy- and deoxy-fluoro oligosaccharides. This interesting piece of work demonstrates that the products of acceptor reactions can be determined not only by the choice of enzyme and acceptor, but by the use of altered donors as well. Interestingly, amylosucrase is not effective at catalyzing oligosaccharide acceptor reactions similar to various dextransucrases [25].

One unique aspect of these glucansucrases is that they are capable of utilizing α-D-glucopyranosyl fluoride as a glucosyl donor in lieu of sucrose [53]. This opens up the possibility of utilizing a wide range of altered glycosyl fluorides as donors, to give many different acceptor products.

Pazur *et al.* [54] isolated from *A. oryzae* a glucosyltransferase which was capable of disproportionating a number of oligosaccharides, including maltose, isomaltose, panose, nigerose and higher oligosaccharides. Later, Pazur and Ando described a similar enzyme from *A. niger* [55]. A more detailed description of this enzyme's products and a proposed

mechanism were subsequently reported [56]. This enzyme usually transfers α-D-glucopyranosyl units from the donor to a 6-OH group on the acceptor, although other linkages may also be formed.

Another example of the use of a chemically modified sucrose analogue as a glucosyl donor has been published recently [57]. In this instance, the D-glucosyltransferase was from *Protaminobacter rubrum* and the donor was 6'-chloro-6'-deoxysucrose. This enzyme usually forms palatinose (6-O-α-D-glucopyranosyl-D-fructose) from sucrose. The use of a sucrose analogue lacking the 6'-OH on fructose enabled these workers to use this enzyme to produce enhanced yields of α-D-glucosylated D-arabinofuranosides and other acceptor products.

D-Fructosyltransferases

We see from the above discussion that the acceptor reactions of glucansucrases encompass a wide variety of acceptor products. However, another type of glycansucrase exists with an equally broad range of acceptors and products. Levansucrase, which synthesizes a β(2-6)-D-fructan from sucrose, has been known for many years to carry out acceptor reactions. Hestrin *et al.* [58] reported the formation of sucrose analogues from raffinose and acceptor sugars by a levansucrase preparation from *Aerobacter levanicum* (now known as *Erwinia herbicola*). This same group subsequently described in great detail the acceptor reactions of levansucrase with a large number of potential acceptors. Of particular interest were the sucrose analogues produced. Acceptor reactions with D-xylose and D-galactose, for instance, gave rise to xylsucrose and galsucrose, respectively [59]. These are analogues of sucrose in which the D-glucosyl moiety has been replaced with a D-xylosyl or D-galactosyl unit. These sugars were also capable of acting as D-fructofuranosyl donors with levansucrase. The acceptor specificity of levansucrase is broader than that of dextransucrase, so a wide variety of compounds can act as acceptors, often in more than one way. Sucrose itself, for example, can be D-fructosylated at position 6 of the D-glucopyranosyl moiety, position 6 of the D-fructofuranosyl moiety and possibly position 1 of the D-fructofuranosyl moiety [59]. Hestrin and Avigad [60] subsequently surveyed a large number of sugars and derivatives in a systematic attempt to learn more about the specificity and mechanism of action of levansucrase. Many sugars were found to act as acceptors, but many of the products were not structurally identified. The formation of galsucrose and its analogues was studied in somewhat greater detail in a subsequent paper [61] and the acceptor reactions of levansucrase were compared with those of dextransucrase and amylosucrase. Amino-sugars can also serve as acceptors for levansucrase [62], as demonstrated by the fact that both D-glucosamine and *N*-acetyl-D-glucosamine gave rise to oligosaccharides in the presence of sucrose with an enzyme preparation from an Aerobacter species.

Other organisms also produce levansucrase, the most notable being *Bacillus subtilis.* A study of the acceptor reactions of its levansucrase showed results similar to those of *E. herbicola* levansucrase [63]. D-Galactose and D-xylose gave rise to galsucrose and xylsucrose, respectively, D-mannose gave rise to the D-mannose analogue and D-fructose gave rise to a series of levan-oligosaccharides. More recently, another type of D-fructosyltransferase from *B. subtilis* has been described which appears to differ somewhat from most levansucrases [64]. This enzyme purportedly produces oligosaccharide products in much higher yields,

I Galpα1-6Glcpα1-2βFruf
2
Glcpα1

II Glcpβ1-4Glcp
2
Glcpα1

III Galpβ1-4Glcp
2
Glcpα1

IV Glcpα1-2βFruf
4
Galpβ1

V Frufβ2[-1Frufβ2]$_n$-1αGlcp n = 0, 1 or 2

Figure 4. Chemical structures of oligosaccharides referred to in the text.

with little or no levan production. The reaction catalyzed is the transfer of a single D-fructofuranosyl unit to any one of a wide variety of acceptors, with little further reaction taking place. Thus, high yields of a single product can be obtained. Its acceptor substrate requirements also appear to be less specific than other, similar enzymes. These traits may make this enzyme more attractive from a practical stand-point, for example, in the synthesis of novel sweeteners.

While levansucrase is produced mainly by bacteria, another type of D-fructosyltransferase can be found in eucaryotic organisms. Plants, especially, often contain a storage polysaccharide known as inulin, which is a β-(2-1)-D-fructan [65]. Enzymes responsible for inulin biosynthesis from sucrose have been isolated from several sources and are known as inulinsucrases. One plant enzyme that has been studied is that from asparagus roots. This D-fructosyltransferase was found to be highly specific for the transfer of a single terminal β-(2-1)-linked D-fructofuranosyl unit to O-6 of the D-glucopyranosyl residue of sucrose-containing oligosaccharides [66]. Levans have also been found in plants, but little is known regarding the mechanism and specificity of most of these plants' enzymes [65].

Fungi also produce D-fructosyltransferases. An enzyme from *Aspergillus niger* has been isolated and characterized [67] and has been used to produce the oligosaccharide sweeteners known together as Neosugar (V). Other fungal D-fructosyltransferases include

those from *Fusarium oxysporum* [68] and *Aureobasidium pullulans* [69]. The *Fusarium*-derived enzyme was investigated for its ability to transfer D-fructosyl units to a number of acceptor sugars and was able to synthesize oligosaccharides from sucrose and D-xylose, but did not utilize D-galactose, D-mannose, arabinose, D-ribose, L-sorbose, L-rhamnose, cellobiose, maltose, melezitose, 2-deoxy-D-glucose, or 6-deoxy-D-glucose as acceptors. This enzyme is apparently much more specific in its acceptor substrate requirements than most D-fructosyltransferases. The action of the D-fructosyltransferase from *A. pullulans* has been studied in some detail, and a mathematical model for its transferase action put forth [70]. A D-fructosyltransferase from the mold *Aspergillus oryzae* was partially purified by Pazur [71] and was found to produce from sucrose a series of oligosaccharides containing D-fructofuranosyl units linked β(2-1), as in the polysaccharide inulin. More recently, Hirayama *et al.* [72] have purified and described the hydrolytic and transferase action of a β-D-fructofuranosidase from *Aspergillus niger.*

Glycohydrolase Transferase Activity

In a broad sense, transglycosylation can be defined as the transfer of any glycosyl unit to an acceptor bearing an -OH group. Hydrolysis is then a special type of transfer in which the acceptor is water. That most enzymes are capable of transferring glycosyl units to more than one acceptor has been recognized for quite some time [22] and is illustrated not only by the hydrolytic activity of such transferases as dextransucrase and levansucrase [73], but also by the glycosyltransferase action of such hydrolytic enzymes as amylase [74], glucodextranase [75] and trehalase [76]. The use of glycosyl hydrolases to catalyze the formation of glycosidic linkages is not new. The review by Edelman [22] cites many instances of glycosyl transfer by invertases from various sources, and so glycosylation by invertase will not be discussed here. However, a few representative examples of glycosyl transfer by other hydrolytic enzymes can be presented. The formation of D-galactosyl and D-mannosyl saccharides is of special interest, due to the widespread occurrence of these sugars in glycoproteins. Both D-galactosidases and galactanase have been used to form D-galactosyl oligosaccharides. The β-D-galactosidase from *E. coli* has been the most studied of these enzymes [77]. It has been shown that this enzyme exhibits a stereochemical preference for the primary hydroxyl group of acceptors. The best acceptors were found to be glycerol and 2-mercaptoethanol, but the formation of glycosidic linkages to other sugars can also occur. In the case of sugar acceptors, a β(1-6) linkage usually is formed. The *E. coli* enzyme catalyzes both transfer reactions from D-galactopyranosides and condensation reactions from free D-galactose. In the case of transferase reactions, the structure of the acceptor markedly affects the relative rates of transfer reactions and hydrolysis; on the other hand, these rates and therefore the relative amounts of acceptor products formed are much less dependent on the structure of the donor. Many studies have been done using o-nitrophenyl-β-D-galactopyranoside as the donor, but the "natural" substrate for this enzyme is presumed to be lactose. Huber *et al.* [78] did study the formation of oligosaccharides from lactose and found that the distribution of products depended on pH, Mg^{2+} concentration, and the anomeric configuration of the donor. One of the products derived from lactose is allolactose (6-*O*-β-D-galactopyranosyl-D-glucose), which is the natural inducer of the lac-operon in *E. coli*. More recently, Huber *et al.* [79] have investigated the acceptor reactions of *E. coli* β-D-galactosidase in the presence of a large number of acceptors and described the structural requirements for acceptor binding and

Table 1. Examples of enzymatic formation of oligosaccharides.

Glycosides formed	Enzyme(s) Trivial name and E.C. No.	Source	Glycosyl donor(s)	Typical linkages[a]	Reference(s)
Part I. D-Glucopyranosides					
β-D-glucopyranosides	β-D-glucosidase 3.2.1.21	almonds	free D-glucose, β-D-glucosides	1-6, others	95
α-D-glucopyranosides	α-D-glucosidase 3.2.1.20	fungi, bacteria, plants	α-D-glucosides, free D-glucose	various	96-103
α-D-glucopyranosides	glucoamylase 3.2.1.3	fungi	free D-glucose, α-D-glucopyranosyl fluoride	1-4	104 75
α-D-glucopyranosides	glucodextranase 3.2.1.70	*Arthrobacter globiformis* I-42	dextran, isomaltodextrins	1-6	75
α-D-glucopyranosides	α-amylase 3.2.1.1	many	starch, maltodextrins	1-4	74, 106, 107
α-isomaltosides	isomaltodextranase 3.2.1.94	*Arthrobacter. globiformis* T-6	dextran, isomaltodextrins	1-6	105
β-cellobiosides	cellulase 3.2.1.4	various bacteria and fungi	cellodextrins	1-4	108, 109
α-maltooligosaccharides	cyclodextrin glucanotransferase 2.4.1.19	bacteria	starch, maltodextrins, cyclodextrins	1-4	111-113
α-D-glucopyranosides	pullulanase, isoamylase	various microbes	starch	1-6, others	114-118
α-D-glucopyranosides	glucosyltransferase	Aspergillus spp.	α-D-glucooligosaccharides	1-6, others	54-56

Glycosides formed	Enzyme(s) Trivial name and E.C. No.	Source	Glycosyl donor(s)	Typical linkages[a]	Reference(s)
α-D-glucopyranosides	glucosyltransferase	*Protaminobacter rubrum*	sucrose, modified sucrose	1-6, 1-5	57
α-D-glucopyranosides	amylosucrase 2.4.1.4	*Neisseria perflava*	sucrose, sucrose analogues	1-4	25
α-D-glucopyranosides	dextransucrase 2.4.1.5	Leuconostoc, Streptococcus spp.	sucrose, sucrose analogues	1-6, 1-2, 1-3, others	30-47, 52
α-D-glucopyranosides	alternansucrase	*Leuconostoc mesenteroides* B1355	sucrose	1-3, 1-6	48
Part II. Other Glycosides					
β-D-fructofuranosides	invertase 3.2.1.26	many	sucrose, raffinose, misc. β-D-fructo-furanosides	various	22
β-D-fructofuranosides	fructosyl-transferase	fungi	sucrose, raffinose, misc. β-D-fructo-furanosides	2-1, 2-6	67-72
β-D-fructofuranosides	levansucrase 2.4.1.10	*Erwinia herbicola,* Bacillus spp.	sucrose, raffinose	2-6, 2-1	58-64
β-D-fructofuranosides	inulinsucrase 2.4.1.9	plants	sucrose & higher d.p. homologues	2-1, 2-6	65, 66
β-D-galactopyranosides	β-D-galactosidase 3.2.1.23	*E. coli,* other microbes	lactose, phenolic β-D-galactopyranosides, free galactose	1-6, others	77-85

Glycosides formed	Enzyme(s) Trivial name and E.C. No.	Source	Glycosyl donor(s)	Typical linkages[a]	Reference(s)
α-D-galactopyranosides	α-D-galactosidase 3.2.1.22	plants	raffinose oligosaccharide, phenolic α-D-galactosides, galactomannans, free D-galactose	1-6, others	88, 89
β-D-galactopyranosides	endo-galactanase	*Penicillium citrinum*	β-galactans	1-4, others	90, 91
α-D-mannopyranosides	α-D-mannosidase 3.2.1.24	*Canavalia ensiformis*	free D-mannose	1-4, 1-6, others	92
β-D-xylopyranosides α-L-arabinofuranosides	β-D-xylosidase 3.2.1.37	*Penicillium wortmanni*	β-D-xylopyranosides, α-L-arabinofuranosides	various	93
β-chitobiosides	chitinase 3.2.1.14	*Nocardia orientalis*	chitin oligosaccharides	1-4	120

[a] Linkages formed often depends on acceptor structure.

reactivity. Subsequently, other workers have gone on to describe the structures of some of the acceptor products from this enzyme [80, 81]. There is still an active interest in the use of this enzyme for the synthesis of specific oligosaccharides, and some improvements have been made towards increasing yields [82] and altering specificity [83]. Amino-sugars may also act as acceptors for this enzyme [84]. One example of an oligosaccharide containing 2-acetamido-2-deoxy-D-glucose produced enzymatically in this way was recently described by Hedbys *et al.* [85].

Other organisms also produce β-D-galactosidases and most of these are known to carry out transferase reactions. For example, Toba *et al.* [86] found that the β-D-galactosidase from *Aspergillus niger* produced twenty or more oligosaccharides from lactose. They also compared the acceptor reactions of this enzyme with those from several other organisms. One interesting observation has been that the reactivity of a β-D-galactosidase from *Bacillus circulans* towards acceptors was increased by immobilization or cross-linking with glutaraldehyde [87].

α-D-Galactosidases are widespread in the plant kingdom, where they play a role in the metabolism of oligosaccharides of the raffinose family. While the use of these enzymes for the synthesis of oligosaccharides has not received as much attention as the β-D-galactosidases, their acceptor specificity has been studied [88]. Recent research has focused on producing large amounts of α-galactosidases for industrial uses [Porter JE, Ladisch MR and Herrmann K (1989), personal communication].

Like most glycosidases, the α-D-galactosidases catalyze both transferase and condensation reactions. In general, the acceptor specificity favors the formation of glycosidic linkages with primary -OH groups. Thus, the product when D-glucose is the acceptor is usually melibiose, and when sucrose is the acceptor, the main product is usually raffinose. However, other products may also be formed. For instance, the enzyme from *Pycnoporus cinnabarinus* yields no fewer than six different oligosaccharides from raffinose [89].

Although the transferase activity of endo-glycohydrolases is less commonly reported than that of exo-hydrolases, it does occur. One example of this is the transferase action of an endo-galactanase from *Penicillium citrinum* [90], whereby D-galactosyl units are transferred to various acceptor molecules. Glycerol was the best acceptor tested and the major products from the reaction between soybean arabinogalactan and glycerol were 2-*O*-β-D-galactopyranosyl glycerol and 4-*O*-β-D-galactopyranosyl-2-*O*-β-D-galactopyranosyl glycerol [91].

Transferase and reversion reactions of D-mannosidases are receiving almost as much attention as those of D-galactosidases lately, due to the prevalence of D-mannosyl units in glycoproteins. A reaction mixture of jackbean α-D-mannosidase and a very high concentration of D-mannose gives rise to at least three different mannobiose isomers, as well as higher oligosaccharides [92]. Such high concentrations of sugars (80% by weight) stabilize the enzyme from heat denaturation, allowing the use of higher reaction temperatures (≈75°C). Another type of glycosylase capable of glycosyl transfer is a β-D-xylosidase from *Penicillium wortmanni* [93]. This enzyme not only catalyzes transfer of β-D-xylopyranosyl units to acceptors, but can also transfer α-L-arabinopyranosyl units. The ability to transfer two different types of sugar units is unusual and makes this enzyme potentially a very useful

means for synthesizing a variety of glycosides. Another β-D-xylosidase has been used to synthesize alkyl-β-D-xylopyranosides *via* D-xylopyranosyl transfer from xylobiose to such alcohols as methanol, ethanol, propanol, butanol and benzyl alcohol [94]. This route to the synthesis of alkyl glycosides holds much promise for the production of these important surfactants.

Due to their biological and commercial importance, the most widely studied enzymes are the glucohydrolases. These include the α- and β-D-glucosidases, amylases, cellulases, and related enzymes. Some of these have already been mentioned in previous sections. Much of the present work on exo-D-glucosidases parallels that being done on the other exo-glycosidases already discussed. For example, β-D-glucosidase from almonds has been used to produce β-D-glucopyranosides from a 90% D-glucose solution at 55°C [95]. A purified β-D-glucosidase from a Streptomyces species has also been shown to produce oligosaccharides by glycosyl transfer reactions [96].

α-D-Glucosidases have also received widespread attention, and many are known to produce oligosaccharides *via* condensation and transfer reactions. Again, this work closely parallels that being done on the other exo-glycosidases, with much of it being carried out by the same groups [97]. Fujimoto and Ajisaka [98] have developed methods for the production of α-D-glucosides of fructose, using an enzyme from yeast, for the purpose of investigating the sweetening properties of these glucosides. α-D-Glucosidases from plants [99-101] and from honeybees [102] have also been used to produce various oligosaccharides and D-glucosides. An α-(1-6)-D-glucosidase from *Streptococcus mitis* is also known to produce various α-linked oligosaccharides by transglucosylation [103]. The work done by Pazur and his colleagues on the action and properties of fungal D-glucosyltransferases has already been mentioned.

Several of the α-D-glucosidases just mentioned show their greatest activity on glycosides and oligosaccharides. Another class of exo-glucosidases also exists which possesses greater activity toward polysaccharides. These include the glucoamylases and glucodextranases, among others. Although they have not been studied in as much detail with regards to their ability to form new glycosidic linkages, some have been investigated. A fungal glucoamylase has been used in a mixed aqueous-organic solvent system to produce several α-linked saccharides of D-glucose [104]. Hehre and his co-workers have used both glucoamylase and glucodextranase to produce oligosaccharides from D-glucopyranosyl fluoride and similar substrate analogues [75].

Arthrobacter globiformis strain T6 produces a somewhat unusual enzyme which hydrolyzes dextran to give mainly isomaltose. This enzyme is also known to catalyze both the condensation of free isomaltose and the transfer of isomaltosyl groups in relatively high yields [105]. The transfer of disaccharide units in this way is potentially quite useful for the production of certain oligosaccharides.

As with other endoglycanases, endoglucanases have been studied less widely in terms of their transferase activity than have the exo-acting enzymes. It has been noted, however, that endoglucanases too are glycosylases, and as such are quite capable of forming as well as breaking glycosidic bonds. Hehre, again, was one of the first to study these enzymes as

reversible glycosylases, and described the mechanistic implications of glycosyl transfer by α-amylases from several different sources [74, 106]. This phenomenon is probably a general one for α-amylases, and has been observed in one of our laboratories with a bacterial α-amylase ([107]; Cote, unpublished). Such reactions, while interesting and useful in the study of the mechanism of these amylases, seem to be of little practical use at this time, and in fact, may even be an undesirable side reaction in the enzymatic conversion of starch. Cellulases [108, 109] and endodextranase [110] have also been observed to catalyze similar transglycosylations.

A more useful type of glycosyl transfer reaction has already been mentioned in our introduction. A number of enzymes have been described which catalyze the production of cyclodextrins from starch [18, 26]. In addition to producing cyclodextrins, these enzymes have also been used to produce various saccharides, mainly through their coupling and disproportionation reactions [111-113]. Other amylolytic enzymes which have been used to produce unusual oligosaccharides *via* transglycosylation include rice debranching enzyme [114], pullulanase [115-117], and bacterial isoamylase [118].

Outlook

As the foregoing examples illustrate, many types of enzymes are available which can be utilized for the synthesis of saccharides *via* glycosyl transfer or condensation reactions. These can, and in at least one case [119] have been combined to produce complex oligosaccharides. The studies of Hehre and others show that glycoside-degrading enzymes, like any other enzymes, can be made to catalyze their reverse reaction under the proper conditions. As new enzymes are discovered, and as more studies are done using immobilized enzymes, non-aqueous or mixed-solvent systems, and engineered enzymes, we can expect to see an increase in our ability to develop enzymic systems for the custom synthesis of a wide variety of useful and biologically active saccharides.

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